Vitamin K2 Is a Mitochondrial Electron Carrier That Rescues Pink1 Deficiency

Article *in* Science · May 2012

DOI: 10.1126/science.1218632 · Source: PubMed

12 authors, including:

R. Meganathan
Northern Illinois University
69 PUBLICATIONS **2,139** CITATIONS

SEE PROFILE

Vanessa A Morais
University of Leuven
54 PUBLICATIONS **1,543** CITATIONS

SEE PROFILE

Vitamin K_2 Is a Mitochondrial Electron Carrier That Rescues Pink1 Deficiency

Melissa Vos *et al.*

Science **336**, 1306 (2012);
DOI: 10.1126/science.1218632

European DWV variants were already present in honey bee populations before the arrival of the mites. Studies in the United Kingdom (*14*) and New Zealand (*24*) have found that DWV infections and colony collapse did not coincide with the arrival and establishment of *Varroa*, but there was with a 1- to 3-year time lag, which we also observed on Hawaii. This lag appears to be the time required for the selection of virus variants adapted to mite transmission.

Recent studies have found no correlation between the presence of *Varroa* and changes in host immune responses (*10*, *25*, *26*), and the common occurrence of time lags between mite introduction and establishment suggests that the increase in DWV titer and reduction in variant diversity cannot be explained by *Varroa*-induced immunosuppression of honey bees (*27*). The apparent lack of association between ABPV, IAPV, and KBV and *Varroa* in this study may reflect the fact that the latter viruses require a longer lag period to become established in *Varroa* than does DWV, although the prevalence of these viruses varies greatly in *Varroa*-infected areas. Further work is required to elucidate the precise role that *Varroa* may have in influencing the prevalence of the range of viruses that infect bees and their role in colony collapse.

Complete viral genome sequencing and experimental infections of honey bees with different DWV strains are required for testing virulence and *Varroa*-associated honey bee colony losses as was seen on Oahu and the Big Island. The current *Varroa*-adapted DWV variants will continue to evolve, and investigations of virus strain differences may explain the different pathologies currently seen globally in honey bee colonies (*7*). Such variants may interact with other pests, pathogens, environmental factors, and regional beekeeping practices, resulting in recent large-scale losses of honey bee colonies (*6*). This study shows that the spread of *Varroa* in Hawaii has caused DWV, originally an insect virus of low prevalence, to emerge. This association may be responsible for the death of millions of colonies worldwide wherever *Varroa* and DWV co-occur.

References and Notes

1. A. R. Blaustein, P. T. J. Johnson, *Nature* **465**, 881 (2010).
2. K. E. Jones *et al.*, *Nature* **451**, 990 (2008).
3. J. K. Waage, J. D. Mumford, *Philos. Trans. R. Soc. London Ser. B* **363**, 863 (2008).
4. R. A. Morse, N. W. Calderone, *Bee Culture* **128**, 1 (2000)
5. S. J. Martin, *J. Appl. Ecol.* **38**, 1082 (2001).
6. D. L. Cox-Foster *et al.*, *Science* **318**, 283 (2007).
7. A. C. Highfield *et al.*, *Appl. Environ. Microbiol.* **75**, 7212 (2009).
8. N. L. Carreck, B. V. Ball, S. J. Martin, *J. Apic. Res.* **49**, 93 (2010).
9. E. Genersch, M. Aubert, *Vet. Res.* **41**, 54 (2010).
10. R. M. Johnson, J. D. Evans, G. E. Robinson, M. R. Berenbaum, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14790 (2009).
11. P. L. Bowen-Walker, S. J. Martin, A. Gunn, *J. Invertebr. Pathol.* **73**, 101 (1999).
12. D. Sumpter, S. J. Martin, *J. Anim. Ecol.* **73**, 51 (2004).
13. S. Gisder, P. Aumeier, E. Genersch, *J. Gen. Virol.* **90**, 463 (2009).
14. S. J. Martin, A. Hogarth, J. van Breda, J. Perrett, *Apidologie (Celle)* **29**, 369 (1998).
15. J. R. de Miranda, E. Genersch, *J. Invertebr. Pathol.* **103** (suppl. 1), S48 (2010).
16. E. Genersch *et al.*, *Apidologie (Celle)* **41**, 332 (2010).
17. D. Tentcheva *et al.*, *Appl. Environ. Microbiol.* **70**, 7185 (2004).
18. A. C. Baker, D. C. Schroeder, *J. Invertebr. Pathol.* **98**, 239 (2008).
19. S. J. Martin, *Am. Bee J.* **150**, 381 (2010).
20. K. M. Roddy, L. A. Arita-Tsutsumi, *J. Hawaiian Pacific Agriculture* **8**, 59 (1997).
21. C. Yue, M. Schröder, S. Gisder, E. Genersch, *J. Gen. Virol.* **88**, 2329 (2007).
22. J. Moore *et al.*, *J. Gen. Virol.* **92**, 156 (2011).
23. C. Yue, E. Genersch, *J. Gen. Virol.* **86**, 3419 (2005).
24. J. H. Todd, J. R. de Miranda, B. V. Ball, *Apidologie (Celle)* **38**, 354 (2007).
25. P. G. Gregory, J. D. Evans, T. Rinderer, L. de Guzman, *J. Insect Sci.* **5**, 7 (2005).
26. M. Navajas *et al.*, *BMC Genomics* **9**, 301 (2008).
27. X. Yang, D. L. Cox-Foster, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7470 (2005).

Acknowledgments: We thank all the Hawaiian beekeepers that participated in this study and D. Jackson of Sheffield University and C. Godfray of Oxford University for comments. S.J.M. and L.B. were funded by a Natural Environment Research Council urgency grant (NE/H013164/1), an Organisation for Economic Co-operation and Development fellowship, and the C. B. Dennis Trust. G.E.B. and M.P. were funded by a grant from the Waterloo foundation with support from Defra and the Welsh Assembly Government. D.C.S. and A.C.H. were funded by the C. B. Dennis Trust. S.N. and E.M.V. were funded by the U.S. Department of Agriculture's National Institute of Food and Agriculture Tropical and Subtropical Agricultural Research (TSTAR) Program (grant no. 2010-34135-21499) and by support from the Hawaii Department of Agriculture. The data are available in the DRYAD depository at http://dx.doi.org/10.5061/dryad.d54cc.

Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6086/1304/DC1
Materials and Methods
Table S1
Figs. S1 to S7
References (*28*–*37*)

22 February 2012; accepted 27 April 2012
10.1126/science.1220941

Vitamin K_2 Is a Mitochondrial Electron Carrier That Rescues Pink1 Deficiency

Melissa Vos,[1,2] Giovanni Esposito,[1,2] Janaka N. Edirisinghe,[3] Sven Vilain,[1,2] Dominik M. Haddad,[1,2] Jan R. Slabbaert,[1,2] Stefanie Van Meensel,[1,2] Onno Schaap,[1,2] Bart De Strooper,[1,2] R. Meganathan,[3] Vanessa A. Morais,[1,2] Patrik Verstreken[1,2]*

Human UBIAD1 localizes to mitochondria and converts vitamin K_1 to vitamin K_2. Vitamin K_2 is best known as a cofactor in blood coagulation, but in bacteria it is a membrane-bound electron carrier. Whether vitamin K_2 exerts a similar carrier function in eukaryotic cells is unknown. We identified *Drosophila* UBIAD1/Heix as a modifier of *pink1*, a gene mutated in Parkinson's disease that affects mitochondrial function. We found that vitamin K_2 was necessary and sufficient to transfer electrons in *Drosophila* mitochondria. *Heix* mutants showed severe mitochondrial defects that were rescued by vitamin K_2, and, similar to ubiquinone, vitamin K_2 transferred electrons in *Drosophila* mitochondria, resulting in more efficient adenosine triphosphate (ATP) production. Thus, mitochondrial dysfunction was rescued by vitamin K_2 that serves as a mitochondrial electron carrier, helping to maintain normal ATP production.

Parkinson's disease (PD) is a common neurodegenerative disorder, and genetic causes of the disease allow us to elucidate the molecular pathways involved (*1*, *2*). Mutations in *pink1*, encoding an evolutionarily conserved mitochondrial kinase, cause PD in humans and mitochondrial defects in model organisms (*3*–*6*). To understand Pink1 function in vivo, we performed a genetic modifier screen in *Drosophila*. Because PD affects the nervous system we screened 193 chemically induced recessive lethal mutants that were selected for defects in neurocommunication (*7*–*9*). We tested dominant modification of $pink1^{B9}$ null mutant flight defects (fig. S1A). Although none of the chemically induced mutants showed dominant flight defects when crossed to a wild-type $pink1^{RV}$ allele, 24 mutants suppressed and 32 enhanced the $pink1^{B9}$ flight defect, such that $pink1^{B9}$ flies failed to fly (fig. S1A).

To reveal the mechanism by which the modifiers affected Pink1, we mapped one of the strongest enhancers that, in combination with $pink1^{B9}$, results in enhanced lethality to *heixuedian* (*heix*). We named this allele $heix^2$ and identified several additional *heix* alleles (fig. S1, B to E) (*10*). To test whether loss of *heix* specifically exacerbated *pink1* phenotypes, we assessed flight, adenosine triphosphate (ATP) levels, and neuronal mitochondrial membrane potential (Ψ_m) (*10*). Heterozygosity for *heix* combined with

[1]VIB Center for the Biology of Disease, 3000 Leuven, Belgium. [2]KU Leuven, Center for Human Genetics and Leuven Research Institute for Neurodegenerative Diseases (LIND), 3000 Leuven, Belgium. [3]Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA.

*To whom correspondence should be addressed. E-mail: patrik.verstreken@med.kuleuven.be

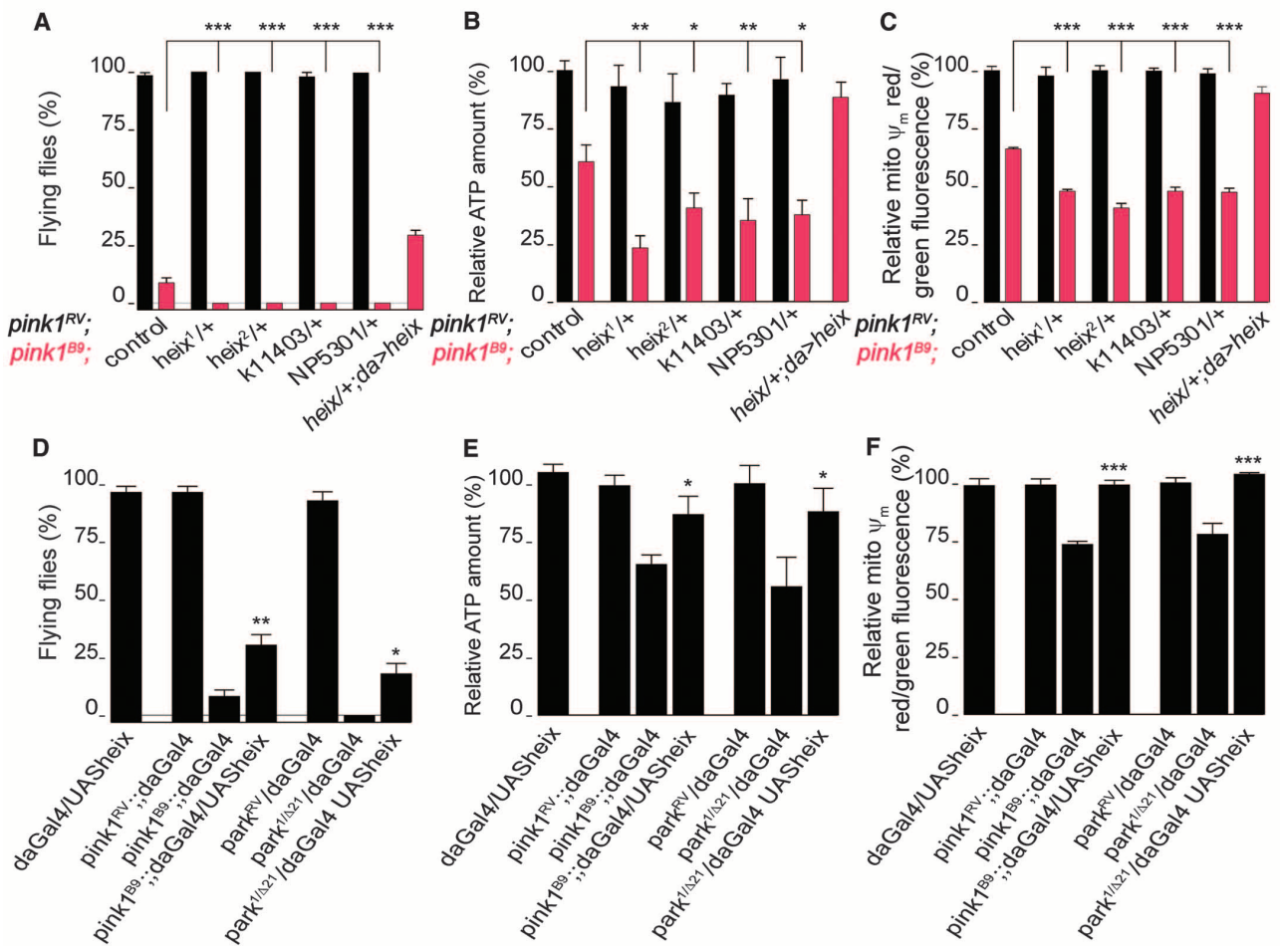

Fig. 1. Identification of *heix* as modifier of *pink1*^{B9}. Enhancement of *pink1*^{B9} phenotypes by heterozygosity for *heix* (pink) compared with *pink1*^{RV} controls heterozygous for *heix* (black) and rescue with *UAS-heix* (*k11403/+;da>heix*); flight ($n > 50$) (**A**), ATP levels ($n = 10$ assays) (**B**), and Ψ_m measurements using the potentiometric dye JC-1. Red aggregate/green monomeric fluorescence ratio measured at third instar neuromuscular boutons; aggregates accumulate at negative Ψ_m ($n = 20$ synapses) (**C**). Suppression of *pink1*^{B9} and *park*$^{1/\Delta 21}$ phenotypes by overexpression of Heix (*da>heix*); flight ($n > 50$) (**D**), ATP levels ($n = 10$ assays) (**E**), and JC-1 red/green fluorescence ratio ($n = 20$ synapses) (**F**). Data are percentage [(A) and (D)] and mean normalized to control [(B), (C), (E), and (F)]. Error bars indicate SEM. Analysis of variance (ANOVA)/Dunnett: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

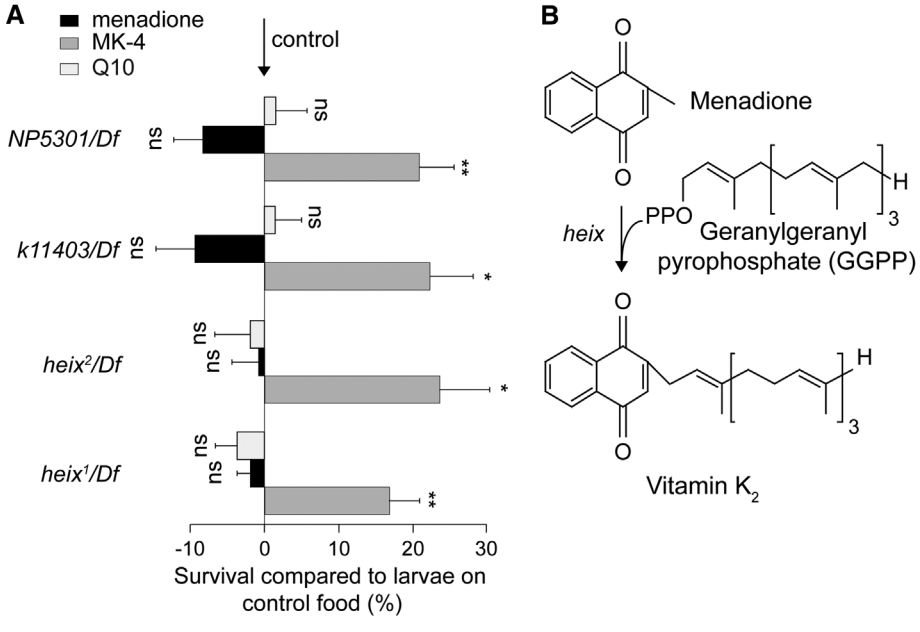

Fig. 2. *Heix* is involved in vitamin K_2 production. (**A**) Improved survival of first instar *heix* mutants placed on MK-4, on ubiquinone (Q10), or on menadione (black) shown as the difference in the percentage of control larvae that survive on control medium ($n = 10$ experiments, each with 10 larvae); that is, about 20% more *heix* mutant larvae survive with vitamin K_2 compared with *heix* mutants on control medium. Error bars, SEM. ANOVA Dunnett: $^*P < 0.05$; $^{**}P < 0.01$. ns, not significant. (**B**) Schematic of the biochemical conversion of menadione to vitamin K_2.

pink1^{RV} did not display defects, whereas it strongly enhanced defects in *pink1*^{B9} null mutants that are rescued by overexpressing Heix (Fig. 1, A to C, and fig. S2). Conversely, overexpression of Heix rescued *pink1*^{B9}-associated defects and those in *parkin*$^{1/\Delta 21}$, which also encodes a PD-associated gene affecting mitochondria (Fig. 1, D and E) (*4*, *5*). Although Heix overexpression does not fully rescue flight, suggesting Pink1 acts in different pathways, the effect is similar in magnitude to previously identified suppressors (*4*, *5*, *11*). Thus, Heix is a dosage-sensitive modifier of *pink1*.

Heix is evolutionary well conserved (fig. S3A), and its bacterial and human homologs, MenA and UBIAD1, harbor a prenyltransferase domain involved in synthesis of vitamin K_2/menaquinone (MK-*n*, *n* indicating the number of prenyl groups) (fig. S3A) (*12*, *13*). In a dendrogram, the three proteins cluster closely, suggesting the enzymes are functionally conserved from bacteria to human (fig. S3B). Feeding *heix* mutants during development with MK-4 but not with menadione, a synthetic precursor that is prenylated to form vitamin K_2, significantly improved their survival rate (Fig. 2, A and B) and other cellular defects (below), whereas ubiquinone (Q-10) feeding did not (Fig. 2A), indicating specificity (*10*). Thus, similar to UBIAD1 and MenA, Heix produces vitamin K_2 in *Drosophila*. We thus tested whether supplementing *pink1*^{B9} with vitamin K_2 alleviated its phenotypes. Because *Escherichia coli* produces vitamin K_2 (MK-8) (*14*), we supplemented minimal medium with living *E. coli* and then placed 1-day-old adult control and *pink1*^{B9} mutant flies on this medium. A significant improvement in flight and ATP levels was observed in *pink1*^{B9} (Fig. 3, A to C). In contrast, *pink1*^{B9} flies on medium with *menA* mutant *E. coli* that barely produce vitamin K_2 (fig. S4A) (*12*) were not rescued (Fig. 3, A to C), whereas *pink1*^{B9} on medium with *menA* mutant *E. coli* complemented with a wild-type *menA* gene (fig. S4A) showed a significant improvement in their flight and ATP defects (Fig. 3, A to C). Thus, vitamin K_2 produced in *E. coli* alleviates *pink1*-related defects.

Next, we placed 1-day-old adult *pink1*^{B9} mutants and controls on MK-4–supplemented or on control medium. Control flies flew normally and did not show altered ATP levels or Ψ_m after feeding with various concentrations of MK-4 (100 to 1000 μM) (Fig. 3, D, F, and G, and fig. S4, B and C). *Pink1*^{B9} on MK-4 showed a dose- and time-dependent improvement of flight, increased ATP amount, and a more negative Ψ_m (Fig. 3, E to G, and fig. S4, B and C). Similarly,

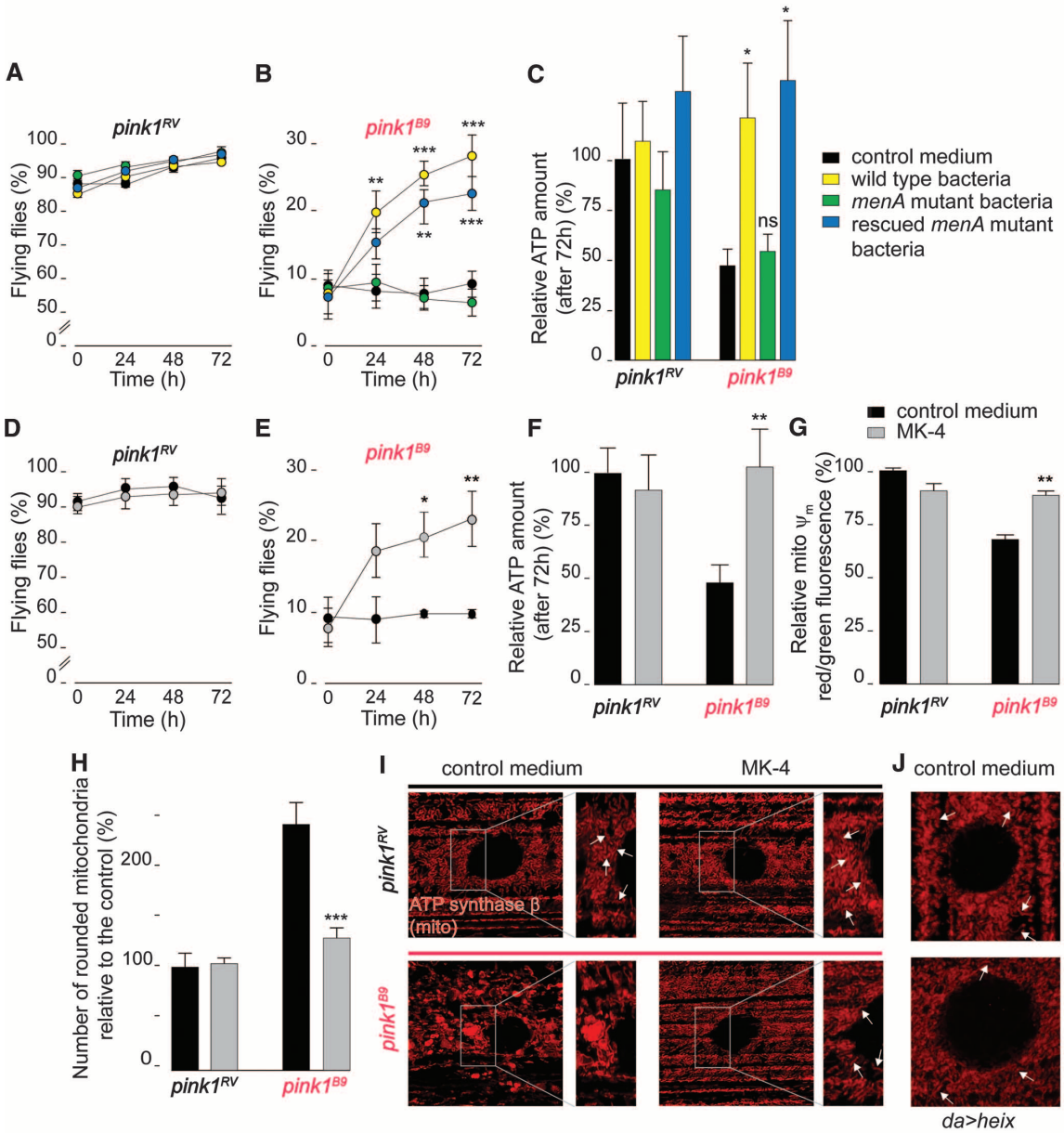

Fig. 3. Vitamin K_2 rescues *pink1*^{B9} mutant phenotypes. Flying ability of *pink1*^{RV} (**A**) and *pink1*^{B9} (**B**) and ATP levels in *pink1*^{RV} and *pink1*^{B9} flies (**C**) that were placed on control medium (black), on medium with *E. coli* that produce vitamin K_2 (MK-8, yellow), or on medium with *menA* mutant *E. coli* that do not produce vitamin K_2 (green) or with *menA* mutant *E. coli* transformed with wild-type *menA* that produce vitamin K_2 (blue) (fig. S4). Flying ability (**D** and **E**), ATP levels (**F**), Ψ_m at neuromuscular boutons determined by using JC-1 red/green ratio (**G**), and mitochondrial morphology in third instar larval muscles (**H** to **J**) in control *pink1*^{RV} and in *pink1*^{B9} on MK-4 containing medium (gray) or control medium (black) [(D) to (I)] or upon overexpression of Heix (*da>heix*) (J). The amount of rounded mitochondria is quantified (H). Arrows are normal mitochondrial structure in (I) and (J). Error bars, SEM. $n > 50$ flies, and data are percentages in (A), (B), (D), and (E); 10 assays in (C) and (F); 20 synapses or muscles in (G) to (J); and mean normalized to control in (C) and (F) to (H). ANOVA/Dunnett: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

park$^{1/\Delta 21}$ mutants showed improved flight, increased ATP levels, and a more negative Ψ_m when placed on MK-4 compared with *park*$^{1/\Delta 21}$ mutants on control medium (fig. S5, A to D). Thus, MK-4 acutely rescued PD-gene-associated mitochondrial and systemic defects in flies.

Larval and adult *pink1*^{B9} and *parkin*$^{1/\Delta 21}$ muscles show enlarged, clumped mitochondria, a defect that is modified by mitochondrial remodeling (*15*). This mitochondrial morphology defect was partially rescued by placing *pink1*^{B9} or *park*$^{1/\Delta 21}$ on MK-4 (Fig. 3, H and I, and figs. S6 and S5, E to I) (*10*) and by overexpressing Heix (Fig. 3J and fig. S5J), indicating that this rescue of mitochondrial morphology was because of a *heix*-dependent vitamin K_2 deficit. We believe that this effect of vitamin K_2 is the result of improved mitochondrial function and not because of an important role for vitamin K_2 in mitochondrial remodeling, because feeding MK-4 to *drp1*$^{1/2}$ or *pink1*^{B9}*;drp1/+* mutants that are defective in mitochondrial fission did not rescue their mitochondrial morphological defects (fig. S7, A and B). Conversely, mitochondrial morphological defects observed in animals expressing RNA interference (RNAi) to electron transport chain (ETC) complex I subunits were alleviated when placed on MK-4 (fig. S7C). Thus, morphological defects that arise because of functional defects in mitochondria can be rescued by vitamin K_2.

In bacteria, vitamin K_2 transfers electrons in the ETC, establishing a proton motive force across the membrane (*16*). Could vitamin K_2 contribute to electron transport in the ETC in mitochondria? Heix-His expressed in fly cells was present in mitochondria (Fig. 4, A and B) (*10*), similar to the human ortholog UBIAD1 (*17*), and vitamin K_2 has previously been detected in mitochondrial fractions (*18*, *19*). We tested the consequence of loss of *heix* on mitochondrial function (*10*) and found that, compared with controls, *heix* mutants showed a less negative Ψ_m and lower ATP levels that could be significantly rescued by rearing *heix* mutants on MK-4 or by reexpression of Heix (Fig. 4C and fig. S8, A and B). Similarly, severe Ψ_m defects in *pink1*^{B9}*;heix/+* were rescued almost to control levels when placed on MK-4 or reexpression of Heix (fig. S8, C and D), and intact mitochondria purified from *heix* mutants showed a significantly reduced capacity to produce ATP in vitro (Fig. 4D). Thus, MK-4 in *heix* mutants is necessary to maintain a negative Ψ_m and to produce ATP.

To determine whether vitamin K_2 is sufficient to facilitate electron transport in mitochondria, we prepared mitochondrial fractions and measured reduction of an artificial electron acceptor, 2,6-dichlorophenolindophenol (DCPIP), downstream of complex II/succinate dehydrogenase (EC 1.3.5.1). In this reaction, succinate is the electron donor and Q-10 the electron carrier (fig. S9) (*20*). When we added MK-4 rather than Q-10, we found that it was also effective at reducing DCPIP, and increasing concentrations of MK-4 resulted in more efficient electron transport (Fig. 4E) (*10*). Compared with MK-4, Q-10 was more effective at reducing DCPIP (Fig. 4F); however, when we placed 1-day-old adult *pink1*^{B9} animals on Q-10 medium, we observed a systemic and mitochondrial rescue that did not exceed that obtained when mutants were reared on MK-4 (fig. S10 and Fig. 3). Direct application of MK-4 to mitochondria purified from *pink1*^{B9} significantly facilitated ATP production (Fig. 4G). Furthermore, time-dependent oxygen consumption of intact mitochondria prepared from *pink1*^{B9} mutants was increased when the flies were first placed on MK-4 (Fig. 4H) (*10*). Thus, vitamin K_2 is sufficient for electron transport downstream of a eukaryotic ETC complex, resulting in improved mitochondrial oxygen

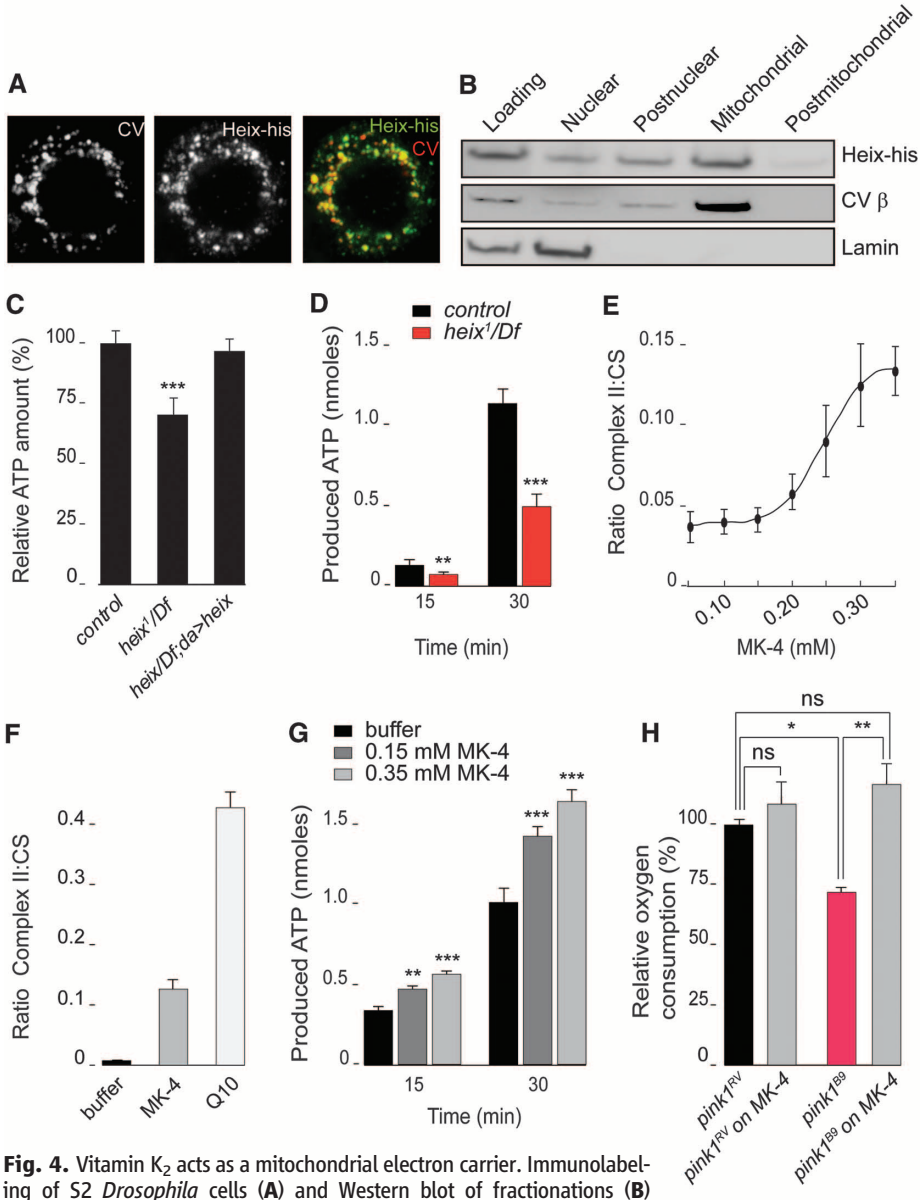

Fig. 4. Vitamin K_2 acts as a mitochondrial electron carrier. Immunolabeling of S2 *Drosophila* cells (**A**) and Western blot of fractionations (**B**) transfected with Heix-His and labeled with anti-His and anti–ATP synthase β (A). (**C**) ATP levels of *heix*^{1}*/Df* and *heix/Df;da>heix* ($n = 8$). (**D**) Time-dependent ATP production in mitochondria isolated from *heix*^{1}*/Df* mutant larvae ($n = 8$). (**E**) Measurements of complex II activity [normalized to citrate synthase activity (CS)] using a dose-response of MK-4 as electron carrier ($n = 4$). (**F**) Measurements of complex II activity using ubiquinone (0.13 mM; Q-10, light gray) or MK-4 (0.35 mM, gray) as electron carrier ($n = 4$). (**G**) Time-dependent ATP production in mitochondria isolated from *pink1*^{B9} mutant flies. Reactions are either not supplemented with MK-4 (black) or supplemented with 0.15 mM (dark gray) or 0.35 mM MK-4 (light gray) ($n = 8$). (**H**) Adenosine diphosphate–stimulated complex I–driven respiration rate (oxygen consumption) in mitochondria isolated from *pink1*^{RV} controls and *pink1*^{B9} mutants placed for 72 hours on MK-4 medium or on control medium. Mitochondria from vitamin K_2-fed mutant flies consume oxygen faster. Data are mean normalized to controls in (C) and (H) or mean in (D) to (G). Error bars, SEM. ANOVA/Dunnett: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

consumption and energy production, particularly in *pink1*^{B9} mutants.

Our data predict that vitamin K_2 may also alleviate the defects in other conditions that impair mitochondrial function. We thus placed flies that express RNAi to different complex I components, *sbo* mutants that produce less ubiquinone, and rotenone-treated animals on control medium or on MK-4 medium and assessed Ψ_m and ATP levels. MK-4 rescued the mitochondrial defects in all of these flies (fig. S11, A and B). These results thus support a role for vitamin K_2 in the transport of electrons in eukaryotic mitochondria to produce ATP, similar to its role in prokaryotic membranes (*16*), suggesting that vitamin K_2 serves a conserved function in prokaryotes and mitochondria. Because mitochondria are involved in aging (*21*), we reared aged flies on MK-4 or overexpressed Heix but did not observe a significant rescue in mobility (fig. S11C). We surmise that long-lasting compensatory changes may be more important in these situations.

Human *UBIAD1* mutations may also affect mitochondrial function. Electron microscopic analyses of corneal samples from Schnyder's crystalline corneal dystrophy patients who harbor mutations in the *UBIAD1* gene (*22*–*24*) indicate cystic swelling of mitochondria, but the nature for this defect is unknown (*25*). Heix/UBIAD1 produces vitamin K_2, and in our studies vitamin K_2 rescued mitochondrial defects in numerous conditions that affect mitochondrial function. Vitamin K_2 was even effective at improving systemic locomotion defects in fully developed adult *pink1* and *parkin* mutant flies. Vitamin K_2 did not affect mitochondrial remodeling directly, but, by increasing ETC efficiency, it contributed to the proton motif force that facilitates ATP production, similar to ubiquinone (*26*). Vitamin K_2 may thus constitute a promising compound to treat mitochondrial pathology, also in PD patients suffering from Pink1 or Parkin deficiency.

References and Notes

1. M. Vila, S. Przedborski, *Nat. Med.* **10** (suppl.), S58 (2004).
2. W. Mandemakers, V. A. Morais, B. De Strooper, *J. Cell Sci.* **120**, 1707 (2007).
3. E. M. Valente *et al.*, *Science* **304**, 1158 (2004); 10.1126/science.1096284.
4. I. E. Clark *et al.*, *Nature* **441**, 1162 (2006).
5. J. Park *et al.*, *Nature* **441**, 1157 (2006).
6. V. A. Morais *et al.*, *EMBO Mol. Med.* **1**, 99 (2009).
7. P. Verstreken *et al.*, *Neuron* **63**, 203 (2009).
8. P. R. Hiesinger *et al.*, *Cell* **121**, 607 (2005).
9. V. Uytterhoeven, S. Kuenen, J. Kasprowicz, K. Miskiewicz, P. Verstreken, *Cell* **145**, 117 (2011).
10. Materials and methods are available as supplementary materials on *Science* Online.
11. S. Vilain *et al.*, *PLoS Genet.* **8**, e1002456 (2012).
12. K. Suvarna, D. Stevenson, R. Meganathan, M. E. S. Hudspeth, *J. Bacteriol.* **180**, 2782 (1998).
13. K. Nakagawa *et al.*, *Nature* **468**, 117 (2010).
14. J. M. Conly, K. Stein, L. Worobetz, S. Rutledge-Harding, *Am. J. Gastroenterol.* **89**, 915 (1994).
15. H. Deng, M. W. Dodson, H. Huang, M. Guo, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 14503 (2008).
16. E. C. C. Lin, D. Kuritzkes, in Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1987), pp. 202–221.
17. M. L. Nickerson *et al.*, *PLoS ONE* **5**, e10760 (2010).
18. H. H. Thijssen, M. J. Drittij-Reijnders, *Br. J. Nutr.* **72**, 415 (1994).
19. Y. Usui *et al.*, *J. Chromatogr.* **489**, 291 (1989).
20. B. de Paepe *et al.*, *Pediatr. Res.* **59**, 2 (2006).
21. S. L. Hebert, I. R. Lanza, K. S. Nair, *Mech. Ageing Dev.* **131**, 451 (2010).
22. A. Orr *et al.*, *PLoS ONE* **2**, e685 (2007).
23. V. S. Yellore *et al.*, *Mol. Vis.* **13**, 1777 (2007).
24. J. S. Weiss *et al.*, *Invest. Ophthalmol. Vis. Sci.* **48**, 5007 (2007).
25. A. Garner, R. C. Tripathi, *Br. J. Ophthalmol.* **56**, 400 (1972).
26. S. McCarthy, M. Somayajulu, M. Sikorska, H. Borowy-Borowski, S. Pandey, *Toxicol. Appl. Pharmacol.* **201**, 21 (2004).

Acknowledgments: We thank H. Bellen, J. Park, J. Chung, the Bloomington *Drosophila* stock center, and the Yale *E. coli* stock center. Support was provided by an Agentschap voor Innovatie door Wetenschap en Technologie (IWT)–Vlaanderen to M.V., a European Research Council Starting Grant (no. 260678), Fonds Voor Wetenschappelijk Onderzoek (G074709-G095511-G094011), the Research Fund KU Leuven, a Methusalem grant, KU Leuven, the Hercules and Francqui foundations, and VIB. The data reported in this paper are tabulated in the main paper and in the supplementary materials. B.D.S. is a paid consultant for Janssen Pharmaceutica (Johnson and Johnson) with regard to Alzheimer's disease and Parkinson's disease.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1218632/DC1
Materials and Methods
Figs. S1 to S11
References (*27*–*44*)

3 January 2012; accepted 13 April 2012
Published online 10 May 2012;
10.1126/science.1218632

Actin Network Architecture Can Determine Myosin Motor Activity

Anne-Cécile Reymann,[1] Rajaa Boujemaa-Paterski,[1] Jean-Louis Martiel,[1] Christophe Guérin,[1] Wenxiang Cao,[2] Harvey F. Chin,[2]* Enrique M. De La Cruz,[2] Manuel Théry,[1]† Laurent Blanchoin[1]†

The organization of actin filaments into higher-ordered structures governs eukaryotic cell shape and movement. Global actin network size and architecture are maintained in a dynamic steady state through regulated assembly and disassembly. Here, we used experimentally defined actin structures in vitro to investigate how the activity of myosin motors depends on network architecture. Direct visualization of filaments revealed myosin-induced actin network deformation. During this reorganization, myosins selectively contracted and disassembled antiparallel actin structures, while parallel actin bundles remained unaffected. The local distribution of nucleation sites and the resulting orientation of actin filaments appeared to regulate the scalability of the contraction process. This "orientation selection" mechanism for selective contraction and disassembly suggests how the dynamics of the cellular actin cytoskeleton can be spatially controlled by actomyosin contractility.

Actin filament networks comprise a large variety of different structures. Their spatial organization supports complex cell-shape regulation. The dynamics and mechanical properties of these structures result from the assembly of polarized actin filaments. Filopodia, retraction fibers, and centripetal fibers are built of parallel filaments (*1*, *2*). Stress fibers and transverse arcs have filaments arranged in antiparallel orientations (*3*, *4*). The lamellipodium is a dense array of branched filaments (*5*).

The global architecture of the actin cytoskeleton is maintained through coordinated actions of a large number of regulatory proteins that modulate filament assembly and disassembly (*6*), as well as through contractility driven by myosin motor proteins (*7*). Myosin motor proteins can also promote filament disassembly (*8*). Collectively, these observations have supported a mechanism in which the coupling between myosin contractility and filament disassembly ensures a temporal synchrony between actin retrograde flow at the front and filament disassembly at the rear of migrating cells (*9*).

Central to this coupling mechanism is that filaments are selected for contraction or disassembly, but it is not known what factors determine the response to myosin contractile forces (*10*). Here, we used micropatterning methods to assemble geometrically controlled and polarized actin filament networks (*11*) to evaluate how the overall polarity of actin filament architectures determines their response—reorganization and/or disassembly—to myosin contractile forces.

Actin filament growth on bar-shaped micropatterns covered with the Wiskott-Aldrich syn-

[1]Institut de Recherches en Technologies et Sciences pour le Vivant (iRTSV), Laboratoire de Physiologie Cellulaire et Végétale, Centre National de la Recherche Scientifique (CNRS)–Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA)–Institut National de la Recherche Agronomique (INRA)–Université Joseph Fourier (UJF), Grenoble, 38054, France. [2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.

*Present address: Department of Biochemistry, Weill Cornell Medical College, New York, NY 10065, USA.
†To whom correspondence should be addressed: manuel.thery@cea.fr (M.T.); laurent.blanchoin@cea.fr (L.B.)