ally, $F(r)$ can be calculated by finding all pairs of trees separated by distances between r and $r + \Delta r$ and then determining what proportion of these pairs are the same species.
9. By overall decay, we mean that all pairs of plots were considered together, with similarity evaluated as a function of distance. The similarity-distance function predicts the slope of a power-law species-area curve (*2*), making it a powerful approach to beta-diversity. Other measures of beta-diversity based on species turnover (*22*) have not used the similarity-distance function but are nevertheless closely related theoretically. On the other hand, we lose information by averaging all pairs of plots (at a given distance); this allows the data to be smoothed and provides theoretically relevant numbers, but abrupt transitions due to habitat change would be missed.
10. Adjacent hectares in Panama are more similar because species richness is lower there—79 species/ha, compared with 173 in Peru and 247 in Ecuador.
11. J. C. Nekola and P. S. White [*J. Biogeogr.* **26**, 867 (1999)] linearized the similarity-distance curve by plotting log-Jaccard versus distance. Our data could not be linearized at all scales with this or any other logarithmic transformation. At distances less than 50 km, the decline in similarity we observed was linear with the log of distance (opposite to the Nekola and White transformation), but Nekola and White lacked data at such short scales. At larger distances, the log of similarity declined linearly with distance in our data, as in boreal forest. We took estimates of the natural log of the Jaccard index from plots 10 to 20 km apart and from plots >1000 km apart and calculated a regression.
12. Single soil samples were taken from each of the 15 1-ha plots in Yasuní. Soil pH, nitrogen, phosphorus, sand, silt content, and nine other measures of soil chemistry showed no spatial autocorrelation; only copper content did.
13. J. S. Clark, *Am. Nat.* **152**, 204 (1998).
14. G. Malécot, *The Mathematics of Heredity* (Freeman, San Francisco, CA, 1969).
15. T. Nagylaki, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2932 (1974).
16. Dispersal kernels where seeds have a high probability of dispersing long distances are called "fat-tailed" (*13*). These may have infinite mean or infinite higher moments, meaning empirically that no matter how many distances have been measured, the next might double the estimate of the moment concerned.
17. Seed dispersal distances were estimated from seedfall into 200 seed traps in the BCI 50-ha plot (*23*) with inverse modeling (*24*, *25*). Gaussian dispersal functions fit significantly better than a null model in 65 tree species (*26*).
18. For all comparisons of plots 18 to 20 km apart in Panama, the mean ± SD of F was 0.0090 ± 0.0071 (N = 77 plot pairs). At Yasuní, at 17 to 22 km, it was 0.0100 ± 0.0027 (N = 21), and at Manu, at 15 to 21 km, it was 0.0129 ± 0.0062 (N = 18). The same trend held for larger distances.
19. Typical wet-forest species occurred on an island of andesite toward the dry side of the isthmus (*4*). Consider six plots on BCI, two plots on the andesite 9 to 12 km south of BCI, and four plots on a sedimentary formation 10 to 13 km east of BCI. For BCI versus sedimentary, the mean F was 0.0145 ± 0.0051 (24 comparisons, ± SD); for BCI versus andesite, the mean F was 0.0038 ± 0.0023 (12 comparisons). The latter is lower than the average F between all plots in Ecuador and all plots in Peru, 1367 km apart (F = 0.0092).
20. R. Condit *et al.*, *Science* **288**, 1414 (2000).
21. All three similarity indices (*8*) are sensitive to species' abundances, even though Sørensen and Jaccard are based only on presence-absence data. In a sample as small as 1 ha of diverse forest, many local species are absent, but abundant species are nearly always present. Thus, presence-absence indices are elevated when the same species are dominant at two sites, relative to a situation where the dominant species at one site are rare at the other.
22. S. Harrison, *Ecology* **78**, 1898 (1997).
23. S. J. Wright, O. Calderón, *J. Ecol.* **83**, 837 (1995).
24. E. Ribbens, J. A. Silander, S. W. Pacala, *Ecology* **75**, 1794 (1994).
25. J. S. Clark, M. Silman, R. Kern, E. Macklin, J. HilleRis-Lambers, *Ecology* **80**, 1475 (1999).
26. H. C. Muller-Landau, dissertation, Princeton University, Princeton, NJ (2001).
27. K. E. Harms, R. Condit, S. P. Hubbell, R. B. Foster, *J. Ecol.* **89**, 947 (2001).
28. We thank the Smithsonian Tropical Research Institute for logistical and financial support; the U.S. Department of Defence Legacy Fund and the U.S. Agency for International Development for financial support of the plot network in Panama; and the Andrew W. Mellon Foundation, the John D. and Catherine T. MacArthur Foundation, and the NSF for supporting the plot networks in Peru and Ecuador.

4 October 2001; accepted 26 November 2001

Role of the Myosin Assembly Protein UNC-45 as a Molecular Chaperone for Myosin

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The organization of myosin into motile cellular structures requires precise temporal and spatial regulation. Proteins containing a UCS (UNC-45/CRO1/She4p) domain are necessary for the incorporation of myosin into the contractile ring during cytokinesis and into thick filaments during muscle development. We report that the carboxyl-terminal regions of UNC-45 bound and exerted chaperone activity on the myosin head. The amino-terminal tetratricopeptide repeat domain of UNC-45 bound the molecular chaperone Hsp90. Thus, UNC-45 functions both as a molecular chaperone and as an Hsp90 co-chaperone for myosin, which can explain previous findings of altered assembly and decreased accumulation of myosin in UNC-45 mutants of *Caenorhabditis elegans*.

The motor protein myosin assembles into molecular machines essential for processes such as cell division, cell motility, and muscle contraction through a multistep pathway requiring additional proteins (*1*). UCS proteins (*Caenorhabditis elegans* UNC-45, *Podospora anserina* CRO1, *Saccharomyces cerevisiae* She4p, and *Schizosaccharomyces pombe* Rng3p) are involved in myosin function and contain homologous COOH-terminal domains (*2*–*6*). *Unc-45* and *RNG3* are essential genes whose loss-of-function alleles implicate their gene products in myosin assembly in vivo; substitutions of conserved residues within or near their UCS domains cause defective assemblies of thick filaments during muscle development and of the contractile ring during cell division (Fig. 1A). UNC-45 and Rng3p interact functionally and specifically in vivo with muscle and cytoskeletal myosins, respectively (*2*, *6*, *7*). UNC-45 also contains an NH_2-terminal domain composed of three tetratricopeptide repeat (TPR) motifs and a newly discovered central region. This three-domain configuration is maintained in all UNC-45 animal homologs identified, including those of *Drosophila*, *Xenopus*, zebrafish, mouse, and human (*8*).

TPR motifs are protein-protein interaction modules of 34 amino acids, often found in tandem repeats of 3 to 16 in a diverse set of proteins (*9*). The UNC-45 TPR domain resembles that of Hop (Hsp70/Hsp90–organizing protein) and of protein phosphatase 5 (*2*, *10*, *11*), which bind conserved COOH-terminal sites in the molecular chaperones Hsp70 and/or Hsp90 (*12*, *13*). Full-length UNC-45 and a TPR-deleted construct [TPR(–)] (*11*) were used to pull down endogenous Hsp70 and Hsp90 from Sf9 insect cell lysates. In our study, only full-length UNC-45 complexed with Hsp90 (Fig. 1B), indicating that this interaction required the TPR domain (*11*). Both constructs pulled down Hsp70, suggesting a possible Hsp70 binding site outside the TPR domain or chaperone-client interactions between these proteins. In the presence of only purified proteins, full-length UNC-45, but not TPR(–), was able to pull down recombinant *C. elegans* Hsp90 (Hsp90) (Fig. 1C) (*11*), indicating a direct interaction between the UNC-45 TPR domain and Hsp90. To determine whether the TPR domain preferentially interacts with Hsp90 or Hsp70, the binding of the recombinant UNC-45 TPR domain (TPR) (*11*) to immobilized Hsp90 (*11*) was competed by *C. elegans* Hsp70 or Hsp90 12-oligomer COOH-terminal peptides (Fig. 1D) (*11*). The structure of the MEEVD (*14*) Hsp90 COOH-terminal

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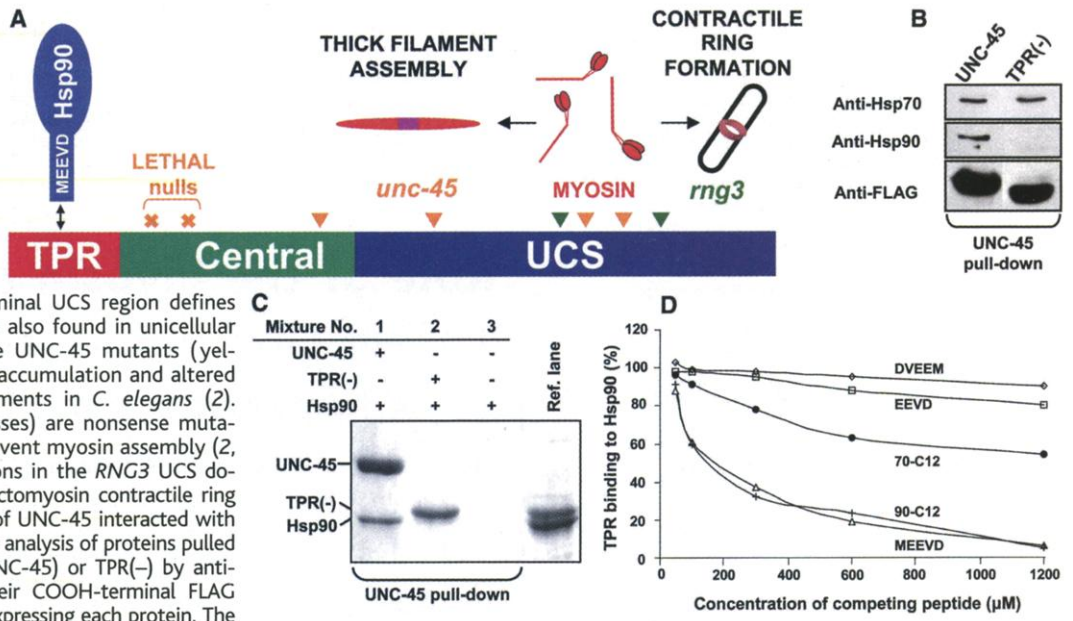

Fig. 1. UNC-45 structure and function relationships. (**A**) *Unc-45* and *S. pombe RNG3* mutations show that UCS proteins are essential in myosin maturation and assembly in vivo. The three-domain structure is conserved in all animal homologs of UNC-45, from *Drosophila* to human (*8*). UNC-45 has an NH_2-terminal TPR domain that closely resembles that of Hsp90-binding proteins. The COOH-terminal UCS region defines the UCS family of proteins and is also found in unicellular eukaryotes. Temperature-sensitive UNC-45 mutants (yellow arrowheads) show decreased accumulation and altered assembly of myosin and its filaments in *C. elegans* (*2*). Lethal *unc-45* alleles (yellow crosses) are nonsense mutations in the central region that prevent myosin assembly (*2*, *7*). Temperature-sensitive mutations in the *RNG3* UCS domain (green arrowheads) disrupt actomyosin contractile ring assembly (*6*). (**B**) The TPR domain of UNC-45 interacted with Hsp90 but not Hsp70. Western blot analysis of proteins pulled down with full-length UNC-45 (UNC-45) or TPR(–) by antibodies to FLAG, which bound their COOH-terminal FLAG epitope tags from Sf9 cell lysates expressing each protein. The membranes were probed with antibodies to Hsp70 and Hsp90. (**C**) SDS-PAGE (Coomassie blue staining) of UNC-45 and TPR(–) (both at 6 μM) pull-downs of Hsp90 (5 μM) at 4°C. TPR(–) and Hsp90 can be resolved in this gel (Ref. lane). (**D**) Surface plasmon resonance analysis of TPR(o) protein (50 μM) binding to immobilized *C. elegans* Hsp90 with various competing peptides. DVEEM and EEVD (*14*) peptides are control peptides; 70-C12 (GGAGGPTIEEVD) and 90-C12 (AEEDASRMEEVD) peptides represent the COOH-terminal amino acids of Hsp70 and Hsp90, respectively, and contain the sequences (GPTIEEVD for Hsp70 and MEEVD for Hsp90) that are necessary and sufficient for binding their corresponding TPR domains (*13*).

pentapeptide is conserved between *C. elegans* and humans, and its specific complex with the Hop TPR 2A domain has been determined crystallographically (*13*). The Hsp90 12-oligomer peptide (90-C12) was as effective as MEEVD at competing with Hsp90 for TPR binding (Fig. 1D), but the Hsp70 12-oligomer peptide (70-C12) showed a slight competitive effect only at high concentrations. The UNC-45 TPR domain therefore appears to bind Hsp90 preferentially.

Although genetic and immunolocalization experiments suggest that nematode body wall myosin interacts with UNC-45 (*2*, *7*, *15*, *16*), there has been no biochemical evidence demonstrating their association. Preliminary experiments showed that recombinant full-length UNC-45 and Hsp90 formed complexes with myosin in *C. elegans* lysates. Direct binding among these proteins was tested with purified components. Binary mixtures containing immobilized myosin and either Hsp90 or UNC-45, and a ternary mixture containing all three proteins, were incubated at 4°C, and the resulting complexes were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2A) (*11*). No interactions between myosin and UNC-45 or Hsp90 were detected. We next evaluated the effect of a 30°C incubation on these mixtures, because this temperature is required in vitro for Hsp90 and its associated co-chaperones to form complexes with other well-studied substrates (*17*) and would enhance hydrophobic interactions. At 30°C, approximately equimolar complexes were detected between myosin and both Hsp90 and UNC-45 in the binary mixtures and among the three proteins in the ternary mixture (Fig. 2B). There was no detectable change in binding of either UNC-45 or Hsp90 to myosin in the ternary mixture as compared to the binary mixtures (Fig. 2B). The ability of Hsp90 to bind myosin directly is similar to its ability to interact with the glucocorticoid receptor in the absence of the co-chaperone Hop (*18*). We next performed pull-down experiments to determine which region of UNC-45 interacted with myosin. We found that TPR(–), like full-length UNC-45, bound myosin. Thus, the myosin-binding site of UNC-45 lies within the central region/UCS domain fragment (Fig. 2C).

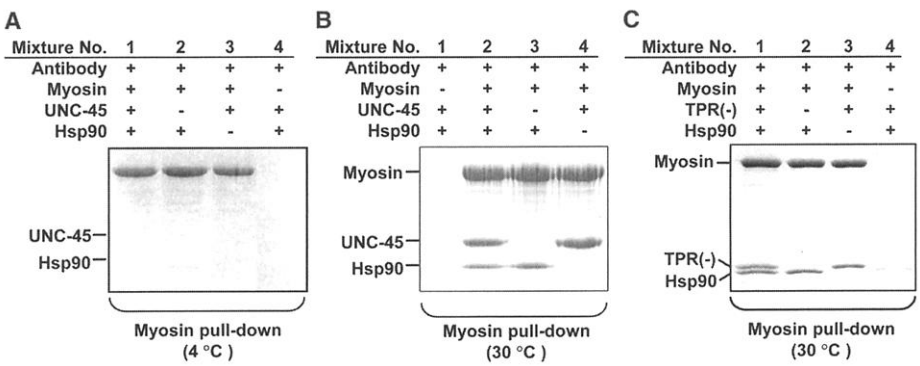

Fig. 2. Myosin interacted with Hsp90 and the COOH-terminal region of UNC-45 in a temperature-dependent manner. All panels are Coomassie blue–stained SDS-PAGE analyses. (**A**) Myosin pull-downs of purified full-length UNC-45 and/or Hsp90 (both at 5.5 μM) at 4°C. There is no detectable interaction of UNC-45 and/or Hsp90. (**B**) Identical reactions to those in (A), except that they were incubated at 30°C for 30 min. Myosin bound both UNC-45 and Hsp90. (**C**) TPR(–) was substituted for full-length UNC-45 under reaction conditions described in (B), with similar results.

Certain myosins and myosin subfragments containing the myosin head are not functional when recombinantly expressed (*24*, *25*), unlike including p23, the large immunophilins, and the Hsp90 protein kinase–targeting subunit Cdc37 (*19*–*21*). Molecular chaperones possess two fundamental biochemical characteristics: (i) the ability to prevent aggregation of partially unfolded proteins and (ii) the ability to maintain partially unfolded proteins in a state competent for refolding (*22*, *23*). UNC-45 demonstrated these characteristics when tested with the well-studied chaperone substrate citrate synthase (*11*, *23*). The chaperone activity of UNC-45 mapped to the central region/UCS domain fragment, the same region responsible for binding myosin (*11*).

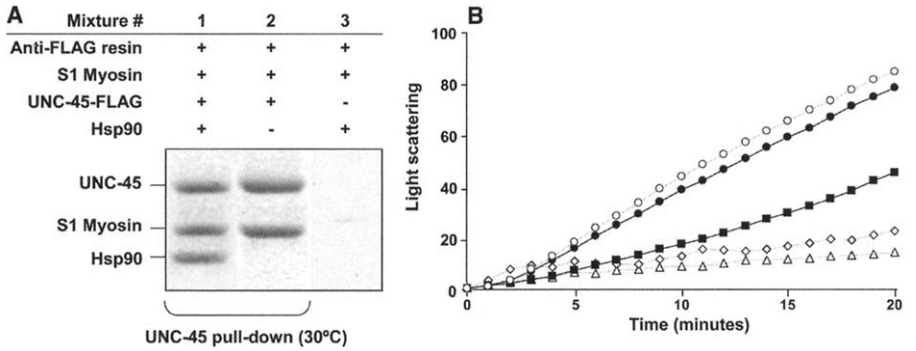

Fig. 3. UNC-45 prevented the thermal aggregation of S1 and formed stoichiometric complexes with Hsp90 and S1. (**A**) SDS-PAGE analysis of S1 (10 μM) and/or Hsp90 (1 μM) interacting with UNC-45 (1 μM) at 30°C for 30 min. (**B**) The aggregation of S1 (1.0 μM) at 43°C was measured by light scattering (320 nm) (*11*) with no additional protein (solid circles), 2.0 μM bovine serum albumin (open circles), 0.5 μM UNC-45 (squares), 1.0 μM UNC-45 (diamonds), or 2.0 μM UNC-45 (triangles).

myosin rod fragments and associated light chains (*26*, *27*), and might require interaction with chaperones. We studied the binding of UNC-45 to myosin heads using myosin subfragment 1 (S1) that had been enzymatically cleaved from intact myosin. Full-length UNC-45, immobilized via its FLAG tag, was used to pull down scallop muscle S1 with or without Hsp90 at 30°C (*11*). UNC-45 formed stoichiometric complexes with S1 and Hsp90 (Fig. 3A). Thus, UNC-45 directly binds the myosin head in addition to Hsp90, which is consistent with the formation of a ternary complex. S1, like citrate synthase, aggregated when incubated at 43°C, but the addition of increasing amounts of UNC-45 reduced S1 aggregation in a concentration-dependent manner (Fig. 3B) (*11*), whereas bovine serum albumin had no effect. Thus, the myosin head is a substrate for the UNC-45 chaperone activity. The biological specificity of this UNC-45 activity toward myosin suggested by the genetic and cell biological experiments may be determined by parts of the molecule that make additional contacts with myosin, as well as by the actual localization of UNC-45 in vivo.

We have shown that UNC-45 binds myosin through its COOH-terminal regions and binds Hsp90 through its TPR domain. Both interactions appeared to be stoichiometric, similar to those of the progesterone receptor with Hsp90 and interacting co-chaperones (*28*). The interaction of UNC-45 with myosin required above-ambient temperatures consistent with a chaperone:substrate relationship. The hypothesis that UNC-45 is a myosin chaperone is supported by the fact that UNC-45 interacted and showed molecular chaperone activity in vitro with the myosin head. Thus, in conjunction with previous genetic studies that implicated UNC-45 in myosin assembly in vivo, our results suggest that the UCS proteins may function as myosin-directed chaperones analogously to Cdc37, which targets Hsp90 to protein kinases and exhibits chaperone activity in vitro (*21*). Previous studies show that the UNC-54 myosin heavy chain in *unc-45* mutant backgrounds acts as a poison for thick filament assembly (*7*), accumulates at 50% lower levels than in the wild type (*2*), localizes abnormally (*2*, *15*), and produces structurally altered thick filaments (*2*). These results may be explained by defects in the chaperone or co-chaperone activity of UNC-45 that lead to altered folding and assembly of myosin.

References and Notes

1. J. M. Barral, H. F. Epstein, *Bioessays* **21**, 813 (1999).
2. J. M. Barral, C. C. Bauer, I. Ortiz, H. F. Epstein, *J. Cell Biol.* **143**, 1215 (1998).
3. V. Berteaux-Lecellier *et al.*, *EMBO J.* **17**, 1248 (1998).
4. R. Jansen, C. Dowzer, C. Michaelis, M. Galova, K. Nasmyth, *Cell* **84**, 687 (1996).
5. B. Wendland, J. M. McCaffery, Q. Xiao, S. D. Emr, *J. Cell Biol.* **135**, 1485 (1996).
6. K. C. Y. Wong, N. I. Naqvi, Y. Iino, M. Yamamoto, M. K. Balasubramanian, *J. Cell Sci.* **113**, 163 (2000).
7. L. Venolia, R. Waterston, *Genetics* **126**, 345 (1990).
8. M. G. Price, J. M. Barral, H. F. Epstein, unpublished observations.
9. G. L. Blatch, M. Lässle, *Bioessays* **21**, 932 (1999).
10. L. Venolia, W. Ao, S. Kim, C. Kim, D. Pilgrim, *Cell Motil. Cytoskeleton* **42**, 163 (1999).
11. For supplementary figures and descriptions of materials and methods, see *Science* Online at www.sciencemag.org/cgi/content/full/295/5555/669/DC1.
12. L. C. Russell, S. R. Whitt, M.-S. Chen, M. Chinkers, *J. Biol. Chem.* **274**, 20060 (1999).
13. C. Scheufler *et al.*, *Cell* **101**, 199 (2000).
14. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
15. H. F. Epstein, J. N. Thomson, *Nature* **250**, 579 (1974).
16. W. Ao, D. Pilgrim, *J. Cell Biol.* **148**, 375 (2000).
17. D. F. Smith, D. B. Schowalter, S. L. Kost, D. O. Toft, *Mol. Endocrinol.* **4**, 1704 (1990).
18. K. D. Dittmar, K. A. Hutchison, J. K. Owens-Grillo, W. B. Pratt, *J. Biol. Chem.* **271**, 12833 (1996).
19. S. Bose, T. Weikl, H. Bügl, J. Buchner, *Science* **274**, 1715 (1996).
20. B. C. Freeman, D. O. Toft, R. Morimoto, *Science* **274**, 1718 (1996).
21. Y. Kimura *et al.*, *Genes Dev.* **11**, 1775 (1997).
22. F. U. Hartl, *Nature* **381**, 571 (1996).
23. J. Buchner, H. Grallert, U. Jakob, *Methods Enzymol.* **290**, 323 (1998).
24. R. Srikakulam, D. A. Winkelmann, *J. Biol. Chem.* **274**, 27265 (1999).
25. E. M. McNally, E. B. Goodwin, J. A. Spudich, L. A. Leinwand, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7270 (1988).
26. A. Delozanne, C. H. Berlot, L. A. Leinwand, J. A. Spudich, *J. Cell Biol.* **105**, 2999 (1987).
27. V. L Wolff-Long, L. D. Saraswat, S. Lowey, *J. Biol. Chem.* **268**, 23162 (1993).
28. H. Kosano, B. Stensgard, M. C. Charlesworth, N. McMahon, D. Toft, *J. Biol. Chem.* **273**, 32973 (1998).
29. We thank H. F. Gilbert for his invaluable help with the chaperone assays and F. Liu and M. G. Price for advice and discussions. Scallop muscle myosin S1 was provided by A. G. Szent-Györgyi. Supported by grants from the Muscular Dystrophy Association and the National Institute of General Medical Sciences (H.F.E.) and a fellowship from the Boehringer Ingelheim Foundation (A.B.).

28 September 2001; accepted 5 December 2001

Activation of Orphan Receptors by the Hormone Relaxin

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Relaxin is a hormone important for the growth and remodeling of reproductive and other tissues during pregnancy. Although binding sites for relaxin are widely distributed, the nature of its receptor has been elusive. Here, we demonstrate that two orphan heterotrimeric guanine nucleotide binding protein (G protein)–coupled receptors, LGR7 and LGR8, are capable of mediating the action of relaxin through an adenosine 3′,5′-monophosphate (cAMP)–dependent pathway distinct from that of the structurally related insulin and insulin-like growth factor family ligand. Treatment of antepartum mice with the soluble ligand-binding region of LGR7 caused parturition delay. The wide and divergent distribution of the two relaxin receptors implicates their roles in reproductive, brain, renal, cardiovascular, and other functions.

Relaxin has diverse actions in the reproductive tract and other tissues during pregnancy (*1*). These actions include promotion of growth and dilation of the cervix, growth and quiescence of the uterus, growth and development of the mammary gland and nipple, and regulation of cardiovascular function. Although binding sites for relaxin have been found in reproduc-