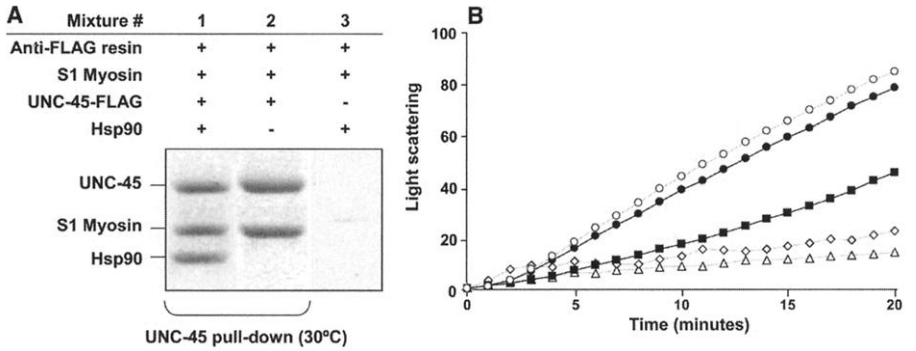

Fig. 3. UNC-45 prevented the thermal aggregation of S1 and formed stoichiometric complexes with Hsp90 and S1. (**A**) SDS-PAGE analysis of S1 (10 μM) and/or Hsp90 (1 μM) interacting with UNC-45 (1 μM) at 30°C for 30 min. (**B**) The aggregation of S1 (1.0 μM) at 43°C was measured by light scattering (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.

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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-

tive tissues (*2*), brain (*3*), and heart (*4*), the nature of the relaxin receptor has not been determined. Prorelaxin, the precursor form of relaxin, has a domain arrangement similar to that of insulin and insulin-like growth factor (IGF) precursors, and several relaxin and insulin-related genes have been identified, including those encoding INSL3 (or Leydig cell relaxin), INSL4, INSL5, INSL6, and relaxin 3 (*5–7*). The abnormal testis descent phenotype of INSL3-null mice (*5, 8*) is similar to that of mice with a disruption of a G protein–coupled receptor (GPCR) encoded by the *GREAT* gene (*9*); this finding suggests that relaxin-related proteins may be ligands for GPCRs. Indeed, relaxin stimulates cAMP production in endometrial, anterior pituitary, and other cells (*1*), an event mediated by GPCRs.

The orphan leucine-rich repeat–containing GPCRs (LGRs) designated as LGR4 through LGR7 are structurally similar to the LGRs for gonadotropins and thyrotropin (*10, 11*). LGR7 can be distinguished from the other three orphan LGRs on the basis of structural motifs and phylogenetic analysis. Moreover, LGR7 likely couples to G_s proteins, because constitutively active LGR7 mutants show ligand-independent cAMP production (*11*). We screened the human genome with LGR7 in search of novel paralogs, and isolated LGR8 (*12, 13*). LGR8 proved to be the human ortholog of the mouse GREAT GPCR and shares about 60% sequence identity with LGR7 (*13*). Phylogenetic analysis also showed that LGR8 is closely related to an orthologous *Drosophila* receptor, DmLGR3, and to the snail LGR (*13*). Similar to the gain-of-function mutants identified for LGR7 ($Asp^{637} \rightarrow Tyr$) (*11*) and the luteinizing hormone (LH) receptor ($Asp^{578} \rightarrow Tyr$), an LGR8 mutant with the same amino acid substitution in transmembrane helix VI conferred a ligand-independent increase in basal cAMP production in transfected human fetal kidney 293T cells (*13*). Thus, LGR7 and LGR8 likely signal through the adenylate cyclase pathway.

Treatment of transfected cells expressing either LGR7 or LGR8 with porcine relaxin resulted in a dose-dependent increase in cAMP production, with median effective concentrations of 1.5 and 5.0 nM, respectively (Fig. 1). In contrast, treatment with structural homologs (insulin, IGF-I, or IGF-II) or with an unrelated peptide (glucagon) was ineffective. These findings indicate that relaxin is a cognate ligand for these two orphan GPCRs and that it activates adenylate cyclases through G_s proteins.

To determine whether the expression of LGR7 and LGR8 is consistent with known relaxin binding sites, we determined their expression patterns (*13*). Reverse transcription polymerase chain reaction (RT-PCR) analysis of 22 different human cDNAs indicated that LGR7 transcript is expressed in the brain, kidney, testis, placenta, uterus, ovary, adrenal, prostate, skin, and heart. However, LGR8 transcript is mainly present in brain, kidney, muscle, testis, thyroid, uterus, peripheral blood cells, and bone marrow. Specific antibodies were generated against the ectodomain of LGR7 (*14*). Immunohistochemical analysis showed that the expression of LGR7 is cell type–specific in different rodent tissues (Fig. 2). In the uterus, LGR7 was expressed mainly in the myometrium and in the epithelial layer of the endometrium. In the vagina and cervix, LGR7 was found in muscularis

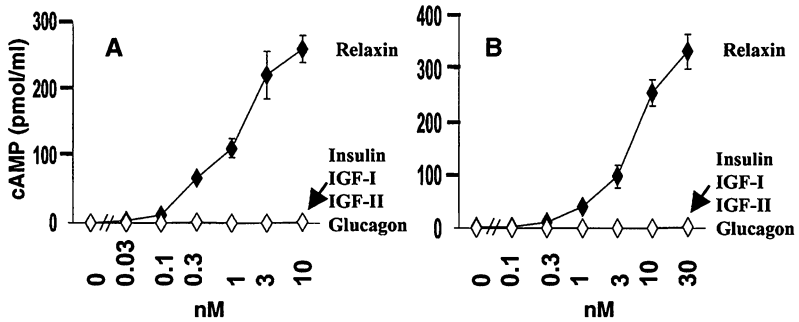

Fig. 1. LGR7 and LGR8 are relaxin receptors. Porcine relaxin stimulated dose-dependent cAMP production in transfected 293T cells (10^5 cells per culture) expressing LGR7 (**A**) or LGR8 (**B**) using the pcDNA3.1-Zeo expression vector (*11*). In contrast, treatment with insulin, IGF-1, IGF-II, or glucagon (a known G_s-coupled receptor activator) had no effect on cAMP production. Treatment with relaxin did not increase cAMP production by nontransfected cells or 293T cells overexpressing related receptors, LH receptor, LGR4, LGR5, or a short splicing variant of LGR7 (*11*). Total cAMP production was measured in triplicate by a specific radioimmunoassay (*10, 28*).

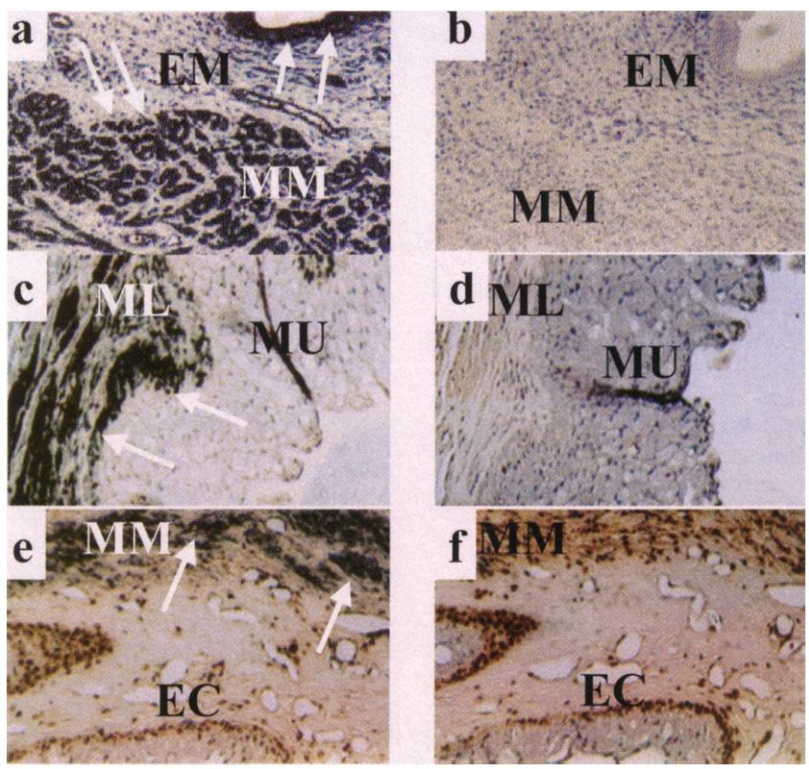

Fig. 2. Tissue distribution of LGR7, as assessed by immunohistochemical analysis in rodent tissues. Uterine tissues were obtained from postpartum rats; vagina and cervix were from rats at 19 days of pregnancy. Specific staining with antibody to LGR7 (*14*) in uterus (**a**), vagina (**c**), and cervix (**e**) is indicated by arrows. Nonimmune serum showed negligible staining in uterus (**b**), vagina (**d**), and cervix (**f**). Abbreviations: EM, endometrium; MM, myometrium; ML, muscularis layer; MU, mucosal layer; EC, endocervix. Immunohistochemical analysis was performed as described (*6*). Magnifications, ×100.

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layers and the myometrium, respectively.

Following an anchored receptor approach previously used to generate soluble ectodomains of the gonadotropin and thyrotropin receptors for use as functional antagonists (*15*), we established stable cell lines expressing a fusion protein that comprised the ectodomain of LGR7 and the transmembrane region of a T cell surface antigen CD8. Upon treatment of cells with thrombin, a thrombin cleavage site located at the LGR7-CD8 junction allowed the release of the soluble ectodomain of LGR7, designated as 7BP. Western blot analysis indicated a single 7BP band of ~60 kD (Fig. 3A). Cross-linking analysis demonstrated the formation of high molecular weight complexes between relaxin and 7BP (Fig. 3B). In contrast, the soluble ectodomain from rat LGR4 (4BP) showed negligible interaction with relaxin.

To determine whether soluble 7BP could serve as a functional antagonist by sequestering relaxin, we simultaneously treated transfected 293T cells expressing LGR7 with relaxin and 7BP (Fig. 4A). Both 7BP and antibodies to porcine relaxin effectively blocked the effects of relaxin. However, cotreatment of relaxin with boiled 7BP or purified 4BP had minimal effect. In addition, the stimulatory effect of relaxin on rat myometrial cells (*16*) was also antagonized by cotreatment with 7BP (Fig. 4B).

The antagonistic action of 7BP was further tested in vivo. Subcutaneous administration of 7BP (500 μg/day) for 4 days (days 17 to 20 after conception) in pregnant mice led to parturition delay by 27 hours (*17*). Most living pups had minimal milk in their stomachs. In antepartum mice, treatment with 7BP also led to the underdevelopment of nipples, as demonstrated by a 29% decrease in nipple size at about 12 hours after parturition (*17*). This finding is consistent with the deficiency of nipple development found in relaxin-null mice (*18*). An earlier study (*19*) also found a disruption of normal delivery in pregnant rats treated with antibodies to relaxin.

The signaling of relaxin through GPCRs is different from that of insulin and IGFs, which involves tyrosine kinase receptors (*20*). The existence of divergent receptor types for relaxin and insulin is consistent with the crystal structures of these ligands (*21*). However, the participation of downstream tyrosine kinases in relaxin signaling could not be ruled out (*22*). The common phenotypes of INSL3-null and GREAT-null mice indicate that INSL3 may be another ligand for LGR8, and possibly for LGR7.

Preterm labor and delivery remain major obstetrical problems. Studies on relaxin receptors could allow the design of agonistic or antagonistic relaxin analogs for the treatment of disorders of labor onset. Relaxin also has a role in regulating pituitary hormone release (*1, 23*), renal vasculature (*24*), and lung and skin remodeling (*25*) as well as in heart failure, angiogenesis, and tumor formation (*26, 27*). The identification of two relaxin receptors with overlapping tissue expression patterns could facilitate our understanding of relaxin actions in diverse physiological and pathological conditions.

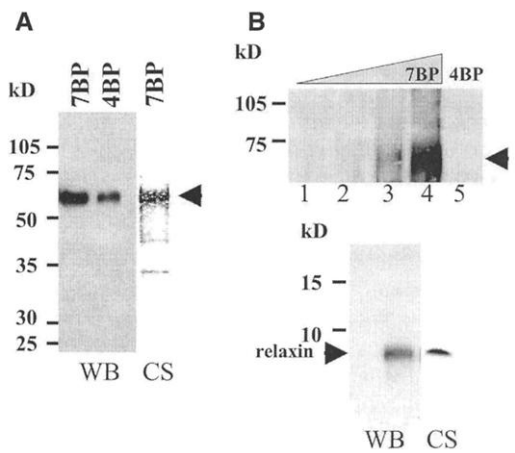

Fig. 3. Relaxin binding to the ligand-binding domain of LGR7. (**A**) A soluble ectodomain of LGR7 (7BP) was detected after Western blot (WB) analysis with anti-FLAG and Coomassie blue staining (CS) of total isolated protein (10 μg) (arrowhead). A homologous domain of LGR4 (4BP) was also generated by a similar approach and detected by Western blot with anti-FLAG (arrowhead). (**B**) Specific interaction of relaxin and 7BP. Upper panel: Purified porcine relaxin (1 μg) (*29*) was preincubated with increasing concentrations (1, 10, 100, and 1000 ng) of purified 7BP for 1 hour and cross-linked with disuccinimidyl suberate at room temperature for 15 min before boiling under denaturing conditions and resolved by 7.5% SDS–polyacrylamide gel electrophoresis. The relaxin-7BP complexes (arrowhead) were detected by antibodies to porcine relaxin (lanes 1 to 4). In contrast, cross-linking of purified 4BP with relaxin showed negligible interaction (lane 5). Lower panel: Purity of the 5.9-kD relaxin is shown by Western blot analysis with antibodies to porcine relaxin (WB) or Coomassie blue staining (CS, 5 μg) under nondenaturing conditions.

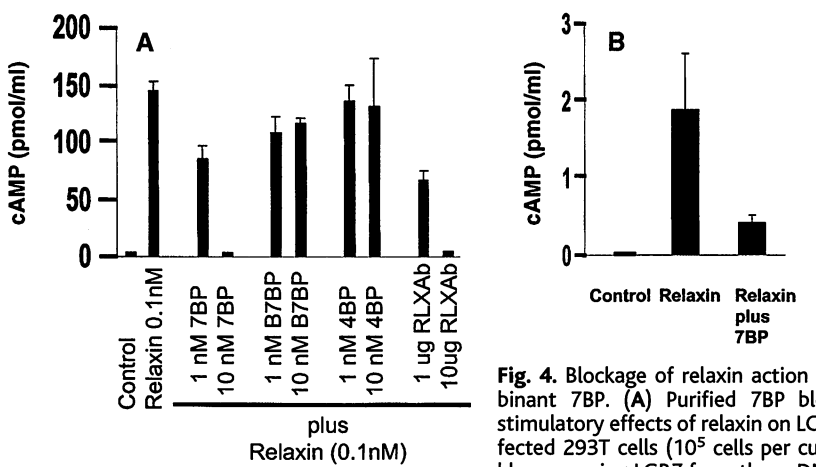

Fig. 4. Blockage of relaxin action by recombinant 7BP. (**A**) Purified 7BP blocked the stimulatory effects of relaxin on LGR7. Transfected 293T cells (10^5 cells per culture) stably expressing LGR7 from the pcDNA3.1-Zeo expression vector were isolated (*14*) and treated (10^5 cells per culture) with 0.1 nM porcine relaxin in combination with different doses of 7BP, boiled 7BP (B7BP), or 4BP for 24 hours under serum-free conditions. In some cultures, antibodies to porcine relaxin (RLXAb) was also included with relaxin. (**B**) Treatment of cultured rat myometrial cells with 7BP blocked the relaxin stimulation of cAMP production. Uterine tissues were obtained from 25-day-old female rats implanted with diethylstilbestrol for 3 days. Myometrial cells were prepared by digestions with trypsin and collagenase as described (*16*). Cells (10^5 per culture) were treated with 1 nM porcine relaxin with or without 7BP for 24 hours.

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13. Supplementary material is available at *Science* Online (www.sciencemag.org/cgi/content/full/295/5555/671/DC1).
14. cDNA for the ectodomain of human LGR7, named as 7BP, was fused in frame with a prolactin signal peptide and the FLAG epitope at the 5′ end and the transmembrane region of T cell surface antigen CD8 followed by a 6-His epitope at the 3′ end. Stable 293T cell lines expressing 7BP in the pcDNA3.1-Zeo

expression vector (Invitrogen) were selected using Zeocin (0.5 mg/ml). To release soluble 7BP anchored on the cell surface, we treated cells with thrombin (10 IU/ml) for 3 days under serum-free conditions. The cleaved 6-His– and FLAG epitope–tagged recombinant 7BP in serum-free conditioned media was purified by sequential nickel and anti-FLAG affinity chromatography. For antibody production, 5 mg of purified 7BP emulsified in Freund's adjuvant were injected into rabbits (Strategic BioSolutions Inc., Ramona, CA). Western blot analysis indicated that the antibody to 7BP does not recognize recombinant LGR8 proteins.

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Plant Biotechnology in China

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A survey of China's plant biotechnologists shows that China is developing the largest plant biotechnology capacity outside of North America. The list of genetically modified plant technologies in trials, including rice, wheat, potatoes, and peanuts, is impressive and differs from those being worked on in other countries. Poor farmers in China are cultivating more area of genetically modified plants than are small farmers in any other developing country. A survey of agricultural producers in China demonstrates that *Bacillus thuringiensis* cotton adoption increases production efficiency and improves farmer health.

Private life-science companies in the industrialized world perform most of the world's agricultural biotechnology research (*1*). Concerns have arisen in developing countries that their scientists and producers can only obtain genes and seeds from foreign companies and that biotechnology research does not focus on the crops that are important to the world's poor farmers. Recently, because of consumer resistance and governmental regulations affecting international trade in genetically modified (GM) products and the rising cost of commercializing new products, private research and development on plant biotechnology is declining, further jeopardizing the little private research that is done on developing country problems (*2*). In contrast, China is accelerating its investments in agricultural biotechnology research and is focusing on commodities that have been mostly ignored in the laboratories of industrialized countries. Small farmers in China have begun to aggressively adopt GM crops when permitted to do so.

The overall goal of this paper is to answer the questions: What is China doing in agricultural biotechnology research? Is China's public-sector–dominated investment strategy efficient? Can China be a source of plant biotechnology for its own farmers and for farmers in the rest of the world?

The first two sections of the paper document China's scientific achievements and research investments. In order to understand the input and output trends of China's plant biotechnology research, in 2000, a two-stage survey elicited information covering approximately 80% of the nation's plant biotechnology research laboratories in nine provinces and two municipalities. In the first stage, based on funding information from the Ministry of Science and Technology (MOST), a list of laboratories that potentially could have been involved in plant biotechnology research was created. Interviews with the research directors identified 35 institutes that conducted research (more than US$30,000) in tissue culture, genetic engineering, marker-assisted selection (MAS), diagnostic technology, microbiology, or other related areas. Twenty-nine institutes provided detailed information on their inputs and outputs for 1999, and 22 institutes provided historic data from 1986. The survey instrument, administered by Chinese Academy of Sciences and Chinese Academy of Agricultural Sciences research staff, contained sections on each institute's total revenues and expenditures, its personnel, investments in biotechnology facilities, and the status of its current and past experiments in the regulatory process. Details of the survey process and a copy of the survey instrument can be found on the *Science* Web site (*3*). The third and fourth sections analyze the economic, environmental, and health impacts of plant biotechnology research using data from a survey of 282 GM cotton farmers in North China.

Although China has spent the last 50 years building the most successful agricultural research system in the developing world—employing more than 70,000 scientists—research in modern plant biotechnology did not begin until the mid-1980s (*4*). Scientists now apply advanced biotechnology tools to the field of plant science, regularly working on the synthesis, isolation, and cloning of new genes and the transformations of plants with these genes. With the initiation of a research program on rice functional genomics in 1997, China's researchers began using AC/DS transposons and T-DNA insertion methods to create rice mutagenesis pools (*5*). Biotechnologists also have initiated functional genomics research for *Arabidopsis*. Our survey of China's laboratories identified over 50 plant species and more than 120 functional genes that scientists are using in plant genetic engineering, making China a global leader in the field.

China's scientists have generated an impressive array of new technologies. From 353 applications between 1996 and 2000, China's Office of Genetic Engineering Safety Administration approved 251 cases of GM plants, animals, and recombined microorganisms for field trials, environmental releases, or commercialization (Table 1, rows 1 and 2). Regulators approved 45 GM plant applications for field trials, 65 for environmental release, and 31 for commercialization (Table 1, rows 3 to 5).

Breakthroughs on food crops that have received little attention elsewhere (>40% of

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