nels, in a manner analogous to those in CNG channels, will be responsible for their proton sensitivity.

REFERENCES AND NOTES

1. R. W. Tsien, P. Hess, E. W. McClosey, R. L. Rosen- 


9. In this paper, we cite the directly measured pH for pure H2O solutions, this value is an overestimate of the actual prepH concentration [L. Pellizzi and E. R. Thornton, J. Am. Chem. Soc. 88, 6531 (1966)]. The actual prepH value of ~logpH 9.0 can be estimated by adding 0.4 units to the cited pH.


11. The measured pH of H2O solutions is 7.74. It was determined by titration of a 30 mM H2O solution with NaOH.

12. Microscopic reversibility requires that all prepH = prepH. The rate constant magnitudes indicate that the system is out of equilibrium because of a "thermodynamic force" of about 1 kT, prepH is the Boltzmann constant and T is the absolute temperature. The observation is explicit because the membrane is held at ~80 mV, and some protons exit to the inside. We discuss the results as if the system is at equilibrium, because our control experiments show that the channel does not depend on the equilibrium conditions. There is a twofold discrepancy between the prepH determined from the amplitude histogram and analysis that determined from the isokinetic rate constants. We attribute this discrepancy to different experimental errors in the two measurements.


20. The DNA construct for the olfactory CNG channel in the high-expression cGMP II vector was kindly pro- 
vided by C. Goulding and S. Siegelbaum of Columbia University (7). RNA was synthesized from Spn 1-in-

21. RPS2 of Arabidopsis thaliana: A Leucine-Rich Repeat Class of Plant Disease Resistance Genes

Andrew F. Bent,* Barbara N. Kunkel,† Douglas Dahlbeck, Kit L. Brown,‡ Renate Schmidt, Jerome Giraudat, Jeffrey Leung, Brian J. Staskawicz§

Plant disease resistance genes function in highly specific pathogen recognition pathways. RPS2 is a resistance gene of Arabidopsis thaliana that confers resistance against Pseudo- monas syringae bacteria that express avirulence gene avrRpt2. RPS2 was isolated by the use of a positional cloning strategy. The derived amino acid sequence of RPS2 contains leucine-rich repeat, membrane-spanning, leucine zipper, and P loop domains. The function of the RPS2 gene product in defense signal transduction is postulated to involve nucleotide triphosphatase binding and protein-protein interactions and may also involve the reception of an elicitor produced by the avirulent pathogen.

Disease resistance gene control recognition of invading pathogens and subsequent activation of defense responses (1, 2). Individual resistance genes are highly specific in function, being effective only against particular strains of a viral, bacterial, fungal, or nematode pathogen. For more than 80 years, crop breeding programs have used disease resistance genes because of their effectiveness in preventing disease and their ease of handling as single Mendelian loci. However, the molecular basis for resistance gene function is only starting to be elucidated.

Many plant pathogens produce specific elicitor compounds that are recognized by resistant plants, thereby triggering active defense responses that curtail pathogen growth (1–3). The pathogen genes that control production of these elicitors are known as avirulence genes, and the activity of a plant resistance gene is dependent on the expression of the specific corresponding avirulence gene in the pathogen. Defense responses observed in infected tissue include production of antimicrobial compounds and cell wall–reinforcing proteins and a localized cell death response known as the hypersensitive response (4). The physiological mechanism by which these processes are activated is not clear, al-

22. Our ability to measure the calculated protonation and deprotonation rates was tested by computer simulation of the open state of a single cationic channel. Assuming scheme 6 with time constants comparable to measured values, a current template with Gaussian noise was generated, digitized filtered, and sampled, then run through the same anal- 

ysis programs. The simulation results showed that the time resolution of the recording system was su- 

cient to give the values of the rates reported here. Misssed events and noise do cause minor deviations in the simulated rates; these deviations are qualita-

tively similar to deviations of the real data from the theoretical curves in Fig. 3, C through E.

23. Cysteine mutants of the pore (M1u-C3α) were gen- 

erated by means of polyclonal rearrangements reaction (Erf- 

comp, Parkin Elmer reagents) and were subcloned into the channel DNA. Mutations were confirmed byideoxy sequencing between the restriction sites (F. Sanger, S. Nolck, A. R. Coulson, Proc. Natl. Acad. Sci. U.S.A. 74, 5483 (1977)).

24. We thank W. P. Jencks, J. Hauge, and P. Chen for helpfull discussions and B. A. Gross, and L. Heggbootham for critical review of the manuscript. Supported by NIH research grant GM47400. M.R. was supported by NSF's Molecular Biophysics Training Grant 5 T32 GM083113.

14 April 1994; accepted 3 August 1994

Fig

the cor e

con

of

R

mi

cos

alo

y

RP

exp

(A)

ti

of

c

tiat

the

Col

line

pro

typ

c

s

1994

1856

SCIENCE • VOL. 265 • 23 SEPTEMBER 1994
though a number of studies indicate that activated oxygen production, the gating of ion channels, and protein phosphorylation may be involved (5). Plant resistance genes have been postulated to encode receptors for the elicitors produced by avirulent pathogens (1, 3), but to date no such receptors have been identified.

Two resistance genes have been characterized at the molecular level. *Hm1* from maize was isolated by the use of a transposon tagging strategy (6). This gene encodes a redox enzyme that confers resistance against specific strains of the fungal pathogen *Cochliobolus carbonum* by degrading the fungal HC toxin, a resistance strategy that is mechanistically distinct from avirulence gene–dependent resistance. The *Pto* gene from tomato, which confers resistance against *Pseudomonas syringae* pv. *tomato* bacteria that express the avrPto avirulence gene, was recently isolated by positional cloning (7). The predicted *Pto* gene product resembles a serine-threonine protein kinase, which suggests (at least for this particular plant-pathogen interaction) that specific protein kinases play a critical role in the induction of host defenses. The cloning of additional resistance genes should greatly facilitate efforts both to understand the molecular basis of resistance and to use biotechnological strategies to improve field crop resistance (1, 2).

The RPS2 resistance gene of *Arabidopsis thaliana* confers resistance against *P. syringae* pv. *tomato* strains that express avirulence gene *avrRpt2* (8, 9). Resistance against *P. syringae* pathogens expressing *avrRpt2* exists not only in *Arabidopsis*, but also in plant species such as soybean and bean (10, 11). Prior genetic mapping had placed *RPS2* in the interval between restriction fragment length polymorphism (RFLP) markers *AtX600* and *AtX557* on *Arabidopsis* chromosome four, approximately 0.5 centimorgan (cM) from *AtX600* (8, 9). To proceed with map-based isolation of the *RPS2* gene, we used *AtX600* as a hybridization probe to identify sets of yeast artificial chromosome (YAC) and cosmid clones that carry contiguous, overlapping inserts of *Arabidopsis* ecotype Col-0 genomic DNA (Fig. 1A) (12). Tightly linked RFLP markers generated from ends of insert DNA from the YAC and cosmid clones (Fig. 1A) were then used to localize *RPS2* to a 0.3-cM interval that corresponds to a region of approximately 200 kb (Fig. 1A).

To identify expressed sequences from the *RPS2* region, we used the cosmid clones 4-6, 4-4, and 7-26 (Fig. 1A) to probe an *Arabidopsis* complementary DNA (cDNA) library (13). Eight different classes of cDNAs were isolated. The inferred gene location for five of these cDNAs is shown in Fig. 1B; three additional cDNAs mapped just outside this region. These eight cDNAs were used to screen for altered transcript production in *rps2* mutants in an effort to determine correspondence between one of the cDNAs and RPS2 (14). However, no alterations were detected (15).

Further efforts to identify *RPS2* used functional complementation of *Arabidopsis* Col-0 *rps2-201/rps2-201* plants. An additional set of overlapping clones from the RPS2 region was constructed with a cosmid vector suitable for Agrobacterium-mediated transformation (16). Three separate transformation methods were used (17-19), including a modified vacuum infiltration procedure that greatly expedited these efforts (20). *RPS2* activity was initially observed in Col-0 *rps2-201/rps2-201* plants transformed with the 18-kb genomic region carried on cosmid pD4 (Fig. 1B). These plants displayed a strong hypersensitive resistance response (HR) when inoculated with *P. syringae* expressing *avrRpt2* but did not develop an HR after inoculation with strains not carrying *avrRpt2*. Disease resistance assays and DNA blots performed on the self-progeny of one pD4 transformant confirmed cosegregation between the resistant phenotype and the introduced DNA (15). Col-0 *rps2-201/rps2-201* plants carrying the wild-type genomic region from cosmid pD4 did not display disease symptoms (Fig. 2). Growth of *P. syringae* expressing *avrRpt2* was restricted in the leaf tissue of Col-0.
The RPS2 sequence predicts a 909-amino acid, 105-kDa gene product (Fig. 4). Support for designation of this open reading frame as RPS2 came both from the complementation experiments discussed above and from sequence analysis of the mutant allele rps2-201. The rps2-201 sequence deviates from that of wild-type RPS2 by a single-nucleotide change that converts Thr668 of this open reading frame to Pro. No other open reading frames are present at the site of the rps2-201 mutation. Additional evidence supporting designation of this gene as RPS2 has been obtained by DNA sequence analysis of mutant alleles rps2-101C, rps2-102C, and rps2-101N (23). No additional sequences with strong similarity to RPS2 were observed when blots of genomic DNA from Arabidopsis, bean, soybean, tomato, green pepper, tobacco, rice, barley, and maize were probed with a 2.8-kb probe encompassing the RPS2 open reading frame (22).

Analysis of the derived amino acid sequence for RPS2 revealed several regions with similarity to known polypeptide motifs (24). Most prominent among these is a region of leucine-rich repeats (LRRs) (25). The LRR motif has been implicated in protein-protein interactions and ligands binding in a diverse array of proteins (25–29). LRRs, for example, form the hormone-binding sites of mammalian gonadotropin hormone receptors (28) and a domain of yeast adenylate cyclase that interacts with the RAS2 protein (26). In RPS2, the LRR domain spans amino acids 503 to 809 and contains 13 repeat units of 22 to 26 amino acids in length; a portion of each repeat resembles the consensus (I/L/V)XXLXX-LXX(IL/X)L (30). Six sequences matching the N-glycosylation consensus [NX(S/T)] (31) were observed in RPS2, and five of these are within the LRR region (Fig. 4). The single-nucleotide difference between functional RPS2 and mutant allele rps2-201 is within the LRR coding region, and this mutation disrupts one of the potential glycosylation sites.

The deduced amino acid sequence for RPS2 carries a second potential protein-protein interaction domain, a leucine zipper (32), at amino acids 30 to 57. This region contains four contiguous heptad repeats that match the consensus sequence (I/R)X-DXXX (30). Leucine zippers facilitate the dimerization of transcription factors by formation of a coiled-coil structure, but no sequences suggestive of an adjacent DNA binding domain (such as a strongly basic region or a potential zinc finger) were detected in RPS2. Coiled-coil regions also promote specific interactions between proteins that are not transcription factors (33), and computer database similarity searches with the region spanning amino acids 30 to 57 of RPS2 revealed the greatest similarity to the coiled-coil regions of numerous myosin and paramyosin proteins.

The sequence GPDCVGGK (30) at deduced amino acids 182 to 189 of RPS2 precisely matches the generalized consensus for the phosphate-binding loop (P loop) of numerous adenosine triphosphate (ATP)– and guanosine triphosphate (GTP)-binding proteins (34). The postulated RPS2 nucleotide binding site is similar to those found in RAS proteins and ATP synthase β subunits (34), but surprisingly is most similar to the published P loop of the cheD gene from plant-colonizing Agrobacterium (35). The presence of this P loop sequence strongly suggests nucleotide triphosphate binding as one aspect of RPS2 function.

A potential membrane-spanning domain is located at amino acids 340 to 360 of the RPS2 gene product, which raises the possibility of membrane localization with the NH2-terminus leucine zipper and P loop domains residing together on the opposite side of the membrane from the LRR region. An orientation in which the COOH-terminal LRR domain is extracellular is suggested by the limited domain.
by the fact that five of six potential N-linked glycosylation sites occur COOH-terminal to the proposed membrane-spanning domain and by the overall more positive charge of the NH2-terminal amino acid residues (31, 36). A number of proteins that contain LRRs are postulated or known to be membrane-spanning receptors in which the LRRs are displayed extracellularly as a ligand-binding domain (27–29).

The plant kingdom contains hundreds of resistance genes that are necessarily divergent because they control different resistance specificities. However, plant defense responses such as production of activated oxygen species, pathogen-respons e (PR) protein gene expression, and the hypersensitive response are common to diverse plant-pathogen interactions (4). This implies that there are points of convergence in the defense signal transduction pathways downstream of initial pathogen recognition and suggests that similar functional motifs will exist among diverse resistance gene products. In support of this hypothesis, RPS2 has been found to share regions of similarity (including the P loop and leucine-rich repeats) with the recently cloned N gene of tobacco (37). RPS2 and N represent a new class of disease resistance genes, as they are not similar to the previously described Hml or Pto genes (6, 7). It is particularly intriguing to note that whereas RPS2 confers resistance against bacterial pathogens, N encodes resistance against isolates of tobacco mosaic virus (3, 37).

Although the molecular function of RPS2 in defense signal transduction remains to be elucidated, the RPS2 sequence predicts a number of testable models. Pathogen avirulence genes have been shown to control the production of resistance gene-specific elicitors of plant defense responses, and resistance genes have long been postulated to encode the receptors for these elicitors (1, 2). Given that LRRs form the extracellular ligand-binding domain of proteins such as the gonadotropin hormone receptors (28), the presence of LRRs and a possible transmembrane domain suggest that the RPS2 gene product may be the receptor of the avrPto2-controlled elicitor. Alternatively, the LRR of RPS2 may function as an intracellular domain that mediates protein-protein interaction in a downstream step of the signal transduction pathway, as is the case for yeast adenylate cyclase (26). The presence of a P loop suggests testing of the RPS2 gene product for nucleotide triphosphate binding activity. Given that leucine zipper and LRR regions are present in the RPS2 sequence, it is highly probable that protein-protein interactions are a key component of RPS2 function. Experiments that examine these models are anticipated to be informative not only regarding RPS2 function, but more generally regarding the molecular nature of plant defense signal transduction.

REFERENCES AND NOTES


Thymus-Neuroendocrine Interactions in Extrathymic T Cell Development

Jin Wang and John R. Klein*

Studies of the development of murine intestinal intraepithelial lymphocytes (IELs) have yielded markedly different results depending on the experimental system used. In athymic radiation chimeras, IELs consist of all subsets found in euthymic mice; adult mice that were athymic at birth have only IELs that are positive for T cell receptor γδ and CD8αα. These differences are resolved by the finding that administration of the neuropeptide thyrotropin-releasing hormone to adult mice thymectomized as neonates leads to the development of all IEL T cells. Thus, a neuroendocrine signal initiated by the thymus during fetal or neonatal life appears to be required for subsequent extrathyphic maturation of gut γδ T cells.

There now is considerable evidence that some, or possibly all, murine intestinal IELs are T cells that have matured independent of the thymus (1-4), thereby constituting the largest group of peripheral extrathyphic T cells. Yet, opinions differ sharply as to which IELs are thymus-dependent and which are thymus-independent (5, 6); those differences primarily reflect the particular experimental system used—that is, whether mice were made athymic as adults (thymic radiation chimera) (2, 3) or whether they were athymic from birth (4, 6). Understanding the basis for those differences is essential for determining which IELs are truly extrathyphic T cells and for understanding which experimental system most accurately reflects the biology of the IELs in normal mice. In that context, several studies have recently reported that engraftment into neonatal athymic mice with fetal thymus or with thymus tissues contained in diffusion chambers (6) leads to the appearance of γδ T cells in the gut. Because in the latter experiments lymphocytes could not enter the engrafted thymus tissue, it was concluded that the thymus stroma directly influences the development of γδ T cells within the gut. As reported here, however, a similar outcome can be achieved by neu- rohormone supplementation in the absence of thymus engraftment, which suggests that although the thymus is involved in some stage of that process, it is not directly responsible for the development of gut γδ T cells.

We isolated IELs from adult athymic radiation chimeras and from adult neonatal thymectomized (NTX) mice (7) and compared them for expression of lymphocyte markers by flow cytometric analyses (8) to IELs from normal euthymic mice (that is, mice with normal thymuses). Because most TCRγδ, CD8αα, and CD5 IELs express Thy-1 (9, 10) and because those populations were of particular interest in our study, the hematopoietic origins of IELs in athymic chimeras were determined with irradiated CBA (Thy-1.2) congenic mice injected with bone marrow from AKR (Thy-1.1) mice. More than 90% of Thy-1+ IELs in athymic chimeras expressed the Thy-1.1 allele, which indicates that they were donor-derived lymphocytes and that they were not residual host cells (Fig. 1). IELs from athymic chimeras (Fig. 2B) expressed all subsets that existed in euthymic mice (Fig. 2A). In those mice, IELs consisted of both TCRγδβ−CD5+ cells and TCRγδ+CD5− cells. Virtually all CD8β+ IELs were Thy-1+ cells, and both CD8αα and CD8αβ IELs were present. CD5 was expressed on some, though not all, TCRγδβ+ IELs; TCRγδ+ IELs were primarily CD5−. Consistent with findings from this (2) and other laboratories (3), T cells were absent in peripheral immune compartments outside the intestine. These findings indicate that despite a reduction in the proportion of some types of IELs in athymic chimeras (for example, CD8β+ and Thy-1+/CD8β+ cells) (Fig. 2B), all IEL subsets nonetheless developed in the absence of the thymus.

In contrast to IELs in euthymic mice and athymic chimeras, IELs in NTX mice consisted of only TCRγδ−CD8αα+ cells (Fig. 2C). Additionally, more than half of the IELs in NTX mice were devoid of markers of mature lymphocytes and, even among the TCRγδ+ IELs, there was a reduction in the proportion of TCRγδ+ IELs in NTX mice compared to the number of IELs from athymic chimeras (Fig. 2). However, the most striking difference between IELs in athymic chimeras and those in NTX mice was the absence of TCRαβ, CD8αβ, Thy-1, and CD5 cells, a finding that was consistent in several IEL isolates from NTX mice. Thus, IELs in mice that are athymic at birth lack γδ T cells.

*To whom correspondence should be addressed.

Department of Biological Science and Menin Bovard Center for Studies in Molecular Biology and Biotechnol- ogy, University of Tulsa, Tulsa, OK 74104, USA.

Fig. 1. Expression of Thy-1.1 and Thy-1.2 on IELs from CBA athymic radiation chimeras infected with AKR bone marrow (7).

1680  SCIENCE • VOL. 265 • 23 SEPTEMBER 1994