A Protein Farnesyl Transferase Involved in Abscisic Acid Signal Transduction in *Arabidopsis*

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The hormone abscisic acid (ABA) modulates a variety of developmental processes and responses to environmental stress in higher plants. A collection of mutations, designated *era*, in *Arabidopsis thaliana* that confer an enhanced response to exogenous ABA includes mutations in the *Era1* gene, which encodes the β subunit of a protein farnesyl transferase. In yeast and mammalian systems, farnesyl transferases modify several signal transduction proteins for membrane localization. The *era1* mutants suggest that a negative regulator of ABA sensitivity must be acted on by a farnesyl transferase to function.

Abscisic acid is a sesquiterpene produced in plants that has been shown to mediate arrest of growth and development in processes such as control of stomatal aperture in leaves and establishment of seed dormancy (*1*). Genes that respond to exogenous ABA applications have been identified (*2*) and secondary messengers such as Ca^{2+} and inositol triphosphates have been implicated in ABA-mediated responses (*3*). However, many of the cellular components and genes involved in ABA reception and downstream transduction have not been well characterized. One approach to identifying such genes involves the isolation of mutants with altered hormonal responses. In *Arabidopsis thaliana*, mutations in several loci, designated *abi*, that reduce the sensitivity of seed to exogenously applied ABA also reduce the degree of dormancy (*4*). Two *Abi* loci encode a putative serine-threonine phosphatase (*5*) and a transcriptional activator (*6*), which suggests that the cellular response to ABA may involve a signaling cascade. Depending on the *Abi* gene mutation, insensitivity to exogenous ABA can vary over a 100-fold range of concentration, which suggests that germination assays with different concentrations of ABA can be used to identify different loci. The *abi* screens, however, only detect reduced sensitivity to ABA and therefore may bias the genes identified. Another genetic approach to distinguish components involved in ABA signaling is the identification of mutations that enhance sensitivity to ABA. Aside from broadening the spectrum of new ABA response mutants, supersensitive screens also identify negative regulators of ABA sensitivity.

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Mutations that confer enhanced response to ABA (*era*) in *Arabidopsis* seeds were identified by their ability to prevent seed germination with low concentrations of ABA that normally permit wild-type seed germination (*7*). Of these, the *era1* mutant class, which includes one transferred DNA (T-DNA) line (*era1-1*, ecotype Wassilewskija) and two neutron-generated mutants (*era1-2* and *era1-3*, ecotype Columbia), was of added interest because this class showed decreased germination efficiency under normal postimbibition conditions (Fig. 1). Because the *abi* class of mutations reduces seed dormancy, mutations that enhance ABA responsiveness should, in principle, be more dormant. Dormancy in *era1* alleles is alleviated by a 4-day chilling period; the efficiency of *era1* germination increases with the length of time the seeds are chilled (Fig. 1). In many plant species, breaking dormancy to allow germination requires vernalization—exposure to moist, low-temperature environments for an extended period (*8*). The germination profile of *era1* mutants may reflect an increased state of ABA-induced dormancy; consequently, these seeds require longer vernalization to germinate. Support for this contention comes from construction of double mutants of *era1* with both ABA biosynthetic (*aba1-1*) and insensitive mutants (*abi1-1* and *abi3-6*). In all cases, the double mutants had reduced dormancy as compared with *era1*, indicating that the increased dormancy observed in *era1* seed is dependent on ABA synthesis or sensitivity (*9*).

The *era1-1* mutation, which is due to a T-DNA insertion (*10*), allowed the isolation of plant genomic regions flanking the insertion (*11*). Using the flanking regions as probes, we isolated wild-type cDNA and genomic clones; sequence analysis of these described a gene encompassing 3.5 kb of genomic DNA (Fig. 2A). The gene contains 13 introns and the T-DNA insertion site in *era1-1* is in intron 8. Southern (DNA) analysis of wild-type DNA, *era1-2*, and *era1-3* probed with *Era1* cDNA revealed that both fast-neutron alleles contain deletions spanning the *Era1* locus (Fig. 2B). Fast-neutron mutagenesis induces small deletions in *Arabidopsis* (*12*), and subsequent genomic analysis with a 14-kb probe that spans the *Era1* locus determined the size of the *era1-2* deletion to be about 7.5 kb and the *era1-3* deletion to be slightly larger (Fig. 2C). Thus, all three *era1* alleles contain DNA disruptions at the same locus, confirming the identity of the *Era1* locus.

Conceptual translation of the longest open reading frame (404 amino acids) in the *Era1* gene produced a protein with a high sequence similarity to yeast, pea, and mammalian protein farnesyl transferase β subunit genes (Fig. 3) (*13*). Farnesyl transferases consist of α and β subunits that dimerize, forming an enzyme that catalyzes the attachment of farnesyl pyrophosphate (15 carbons) to proteins containing a COOH-terminal CaaX motif (*14*), where C designates a cysteine residue, aa is usually aliphatic amino acids,

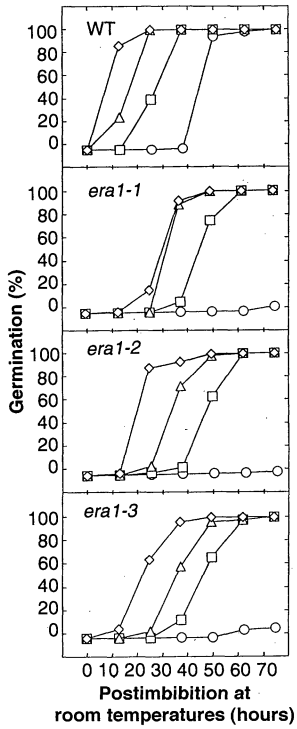

Fig. 1. Germination of the wild type (WT; Columbia) and of *era1* mutants. Mature seeds were chilled for 0 (○), 4 (□), 8 (△), or 12 (◇) days at 4°C in darkness. Germination of seeds was scored as positive when a radicle tip had fully penetrated the seed coat. Each experiment was performed in triplicate and each point represents a germination test of 40 to 50 seeds. Similar results were obtained in all cases. The percentage of germination was determined by dividing the number of seeds that germinated at a given time by the total number of seeds plated.

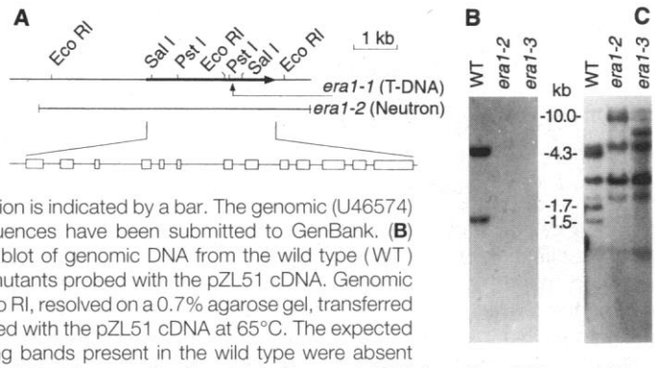

Fig. 2. (**A**) Structure of the *Era1* gene. Exons are denoted by boxes. Introns range from 84 to 367 nucleotides. The site of the T-DNA insertion in the *era1-1* allele is indicated by an arrow and the size of the *era1-2* deletion is indicated by a bar. The genomic (U46574) and cDNA (U44849) sequences have been submitted to GenBank. (**B**) High-stringency Southern blot of genomic DNA from the wild type (WT) and from *era1-2*, *era1-3* mutants probed with the pZL51 cDNA. Genomic DNA was digested with Eco RI, resolved on a 0.7% agarose gel, transferred to nitrocellulose, and probed with the pZL51 cDNA at 65°C. The expected 4.3- and 1.5-kb hybridizing bands present in the wild type were absent from the mutant alleles. (**C**) High-stringency Southern blot of genomic DNA from the wild type and from *era1-2* and *era1-3* mutants probed with a 14-kb genomic fragment encompassing the *Era1* coding region. Southern blots were performed as above. Three hybridizing bands present in wild-type DNA are absent from both mutants (4.3, 1.7, and 1.5 kb).

and X may designate a cysteine, serine, methionine, or glutamine residue. Both plant β subunit genes contain a region of about 50 amino acids near their COOH-terminus (Fig. 3, amino acids 244 to 297) that is absent in yeast and animal β subunit genes.

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                                                                    29
Arab.  MEIQRDKQLD YLMKGLRQLG PQFSSLDAN- --------- ---------
Pea    ..ASTAAETP TPTVSQ.DQW IVE.QVFMIY QLFANIPPNA QSII------
Yeast  .RQRVGRSIA RAKFINTA.L GRKRPVMERV VDIAHVDSSK AIQPLMKELE
Rat    .ASSSSFTTY CPPSSSPVWS EPLY..RPEH ARERLQDDSV ETVTSIEQAK

Arab.  --------- --------- --------- --------- ---------
Pea    --------- --------- --------- --------- ---------
Yeast  TDTTEARTKV LQSVLEITDD EKNIEPALTK EFHKMTLDVA FEISLPPQMT
Rat    VEEKIQEVFS STKFNHLVPR LVLQREKHFH YLKRGLRQ-- ----LTDAYE

                                                                    73
Arab.  -----RFWLC TWILHSIALL G-ETVDDELE SNAIDFLGRC QGSEGGTGGG
Pea    ----..... ...I..... .-.SI..D.. D.TV...N.. .DPN...A..
Yeast  ALDASQ..ML ...AN.LKVM DRDWLS.DTK RKIV.K.FTI SP.G.PF...
Rat    CLDAS..... ......LE.. D-.PIPQIVA TDVCQ..EL. .SPD..F...

                                                                   122
Arab.  PGQLPHIATT YAAVNALVTL GGDKALSS-I NREKMSCFLR RMKDTSGG.R
Pea    ...N..... .....T.I.. ..E.S.A.-. ..N.LTG.M. ...QPN....
Yeast  ....S...S. ...I...SLC DNIDGCMDR. D.KGITQW.I SL.EPN...K
Rat    ...Y....P. .......CII .TEE.TNV-. ....LLQY.Y SL.QPD.S.L

                                                                   171
Arab.  -MHDMGEIDV RACTTAISVA SILNIMDDEL TQGLGDTILS CQTTEGGIGG
Pea    -...E..... ......... .V...L.... IKNV..F... .......LA.
Yeast  TCLEV..V.T .GI.C.L.I. TL...LTE.. .E.VLN.IKN ..N....F.S
Rat    -...VG..V.. .SA.C.A... .LT..ITPD. PE.TAEW.AR ..NW......

                                                                   218
Arab.  EP-GSEAHGG TTTCGL.AM- ILINEVDRLN LDSLMNNAVH RQGV-ENGFQ
Pea    ..-..... ..F..... ...G..N..D .PR.LD.V.F ...K-.C...
Yeast  C.HVD..... ..P.AT.SLA ..RSM-.QI. VEK.LE.SSA ..LQE.R..C
Rat    V.-.M..... ..P....LV ..KK.-RS.. .K..LQ.VTS ..MRF.G...

                                                                   267
Arab.  GRTNKLVDGC TTFWQAAPCV LLQR-LYSTH DHDVHGSSHI SEGTNEEHHA
Pea    ......... .S...GGAVA ....-.H.II .EQMAEA.QF VTVSDAPEEK
Yeast  ..S..... .S..VGGSAA I.EAFG.GQC --------- ---------
Rat    ..C..... .S....GLLP ..H.A.HAQG .PALSM.--- ---------

                                                                   316
Arab.  HDEDDLEDSD DDDDSDEDND EDSVNGHRIH HTSTTINHHM -QLVTDSLGL
Pea    ECL.GTSSHA TSHIRH.GMN .SCSSDVKNI GTNPISEM.Q SEPL.H.IA.
Yeast  ---------- ---------- ---------- ---------- -----.NKHA.
Rat    ---------- ---------- ---------- ---------- -HHM.HQQA.

                                                                   364
Arab.  QRTVLLCSKI -PDGGFRDKP RKPRDFTHTC YCLSGLSVAQ HAMLKDE-DT
Pea    .Q.I....QE -Q...L.... G.R..H..S. .......LC. TS.S.RP-.S
Yeast  RD.I.Y.CQE KEQP.L.... GAHS.....N ...L..A..E SSTSCTPN.S
Rat    .E.I.M.CQC -.A..LL... G.S..... .......I.. -----HPGSG

                                                                   404
Arab.  PPLTRDIMGG YSNLLEPVQL LANIVMDQTN EAIEFFFKAA ----------
Pea    ...RVV..P .......IHP .F.V.L.R.R ..H...SQL- ----------
Yeast  .NNIKCTPDR LIGSSKLTDV NPVYGLPIE. VRKIIHTFKS NLSSPS----
Rat    AM.HDVV..V PE.V.Q.THP VY..GP.KVI Q.TTN.LQKP VFGFEECEDA

Arab.  ----------
Pea    ----------
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Fig. 3. Alignment of the β subunit farnesylation domains from *Arabidopsis* (*Arab.*), pea, yeast, and rat (*19*). The alignments were generated by MACAW and by eye. Residues that are identical to the *Arabidopsis* sequence are indicated with a dot. A dash indicates a blank. The amino acid positions of the *Arabidopsis* gene are indicated on the right-hand side.

Northern (RNA) blot analysis detected accumulation of *Era1* mRNA only in flower buds, which supports a role for this gene in early seed development (*9*). Indeed, extracts from wild-type flower buds had peptide-dependent farnesylation activity in the presence of a farnesyl acceptor (the synthetic peptide GGCCAIM) (*15*) but not when a geranylation (20-carbon) substrate peptide (GGCCAIL) was used (*16*) (Fig. 4). Extracts from *era1-1* and *era1-2* showed no peptide-dependent radioactivity with either the farnesyl or geranyl acceptor peptide (Fig. 4), demonstrating that farnesylation activity is deficient in the two *era1* alleles.

Although ABA-regulated vegetative functions were not determined, the seed phenotypes of *era1* mutants suggest that farnesylation does play a role in embryonic ABA signaling. Animal and yeast signal transduction proteins, including Ras proteins and γ subunits of heterotrimeric GTP-binding proteins (G proteins), are farnesylated

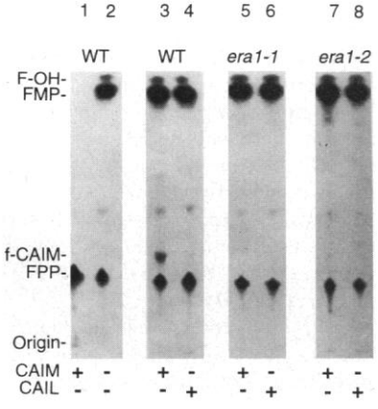

Fig. 4. Farnesylation assay (*15*) on flower bud tissue of the wild type (WT) (Columbia) and of *era1* mutants. Pluses or minuses represent addition or absence of the farnesyl (CAIM) or geranyl (CAIL) peptide target. Lane 1 is boiled (5 min) wild-type extract. F-OH, farnesol; FMP, farnesyl monophosphate; and f-CAIM, farnesylated peptide.

(*16*). Farnesylation in these systems anchors the proteins through hydrophobic interactions with membrane lipids or other proteins (*16*). The increased ABA sensitivity of *era1* mutants and the fact that these are full loss-of-function mutations suggest that a protein or proteins that normally negatively regulate ABA signaling may require farnesylation to function. In some G protein signaling cascades, βγ complexes appear to negatively regulate their effectors (*17*) Perturbed farnesylation could prevent βγ complexes from properly acting as negative regulators and this in turn could cause a supersensitive phenotype. Similarly, in mammalian light reception, attenuation of receptor activation requires farnesylation of the negative regulator rhodopsin kinase (*18*). Whatever the target or targets of ERA1 in ABA signaling, the relatively normal growth and development of *era1* mutant plants suggest either that farnesylation is active only in ABA-mediated cellular responses or that separate farnesylation activities for other signaling pathways exist in plants.

REFERENCES AND NOTES

1. J. A. D. Zeevaart and R. A. Creelman, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 439 (1988); P. M. Chandler and M. Robertson, *ibid.* **45**, 113 (1994).
2. K. Skriver and J. Mundy, *Plant Cell* **2**, 503 (1990).
3. M. R. Blatt, G. Thiel, D. R. Trenthan, *Nature* **346**, 766 (1990); S. Gilroy, N. D. Read, A. J. Trewavas, *ibid.* **346**, 769 (1990); J. I. Schroeder and S. Hagiwara, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9305 (1990); H. R. Irving, C. A. Gehring, R. W. Parish, *ibid.* **89**, 1790 (1992).
4. M. Koornneef and C. M. Karssen, in *Arabidopsis*, E. Meyerowitz and C. Somerville, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994), pp. 313–334; R. R. Finkelstein, *Plant J.* **5**, 765 (1994).
5. J. Leung *et al.*, *Science* **264**, 1448 (1994); K. Meyer, M. P. Leube, E. Grill, *ibid.*, p. 1452.
6. F. Parcy *et al.*, *Plant Cell.* **6**, 1567 (1994).
7. Wild-type seeds show a progressive inhibition of germination with increasing concentrations of ABA, with full inhibition occurring above 1.2 μM. An ABA concentration of 0.3 μM was chosen for screening mutagenized seed populations because this concentration of hormone had no inhibitory effect on wild-type germination. From about 110,000 fast-neutron and 59,000 T-DNA–mutagenized seed pools, 1800 putative mutant lines were identified. In the next generation, 22 lines gave good germination with minimal media and clear supersensitivity to 0.3 μM ABA. Of these, five neutron-induced and two T-DNA–induced mutant lines showed recessive Mendelian inheritance with respect to ABA supersensitivity and defined the three complementation groups *era1*, *era2*, and *era3* (*9*).
8. J. M. Baskin and C. C. Baskin, *Can. J. Bot.* **50**, 277 (1971).
9. S. Cutler, M. Ghassemian, D. Bonetta, S. Cooney, P. McCourt, data not shown.
10. The *era1-1* mutation was scored for cosegregation with the T-DNA disruption by following kanamycin resistance [the neomycin phosphotransferase gene (NPTII) is encoded on the T-DNA vector]. The 108 F_2 seeds that were selected for supersensitivity to ABA all scored positive for kanamycin resistance. Individual F_3 seed families from 120 randomly selected F_2 parents showed absolute linkage of the T-DNA insertion and the ABA-supersensitive phenotype. Zero recombinants indicate with 95% probability that the

T-DNA insertion is within 1.3 map units of the *Era1* gene.

11. Genomic Southern blots of *era1-1* DNA digested with Eco RI and probed with right-border (RB) T-DNA produced three bands (13, 7.0, and 8 kb) that could be accounted for by a contiguous double insertion of T-DNA in which one of the copies has been inverted to give two flanking RB regions attached to plant genomic DNA. Subsequent analysis with other restriction enzymes verified that the 7- and 8-kb bands contained the insertion points of T-DNA and flanking plant DNA. DNA pools enriched for these fragments were ligated to the λ-ZAPII arms according to the manufacturer's instructions (Stratagene) and five positive plaques were identified that hybridized with the RB probe. Two plasmids (pSC10 and pSC11), each containing about 1.2 kb of T-DNA attached to different flanking regions, detected a single Eco RI 1.5-kb fragment in wild-type DNA by Southern analysis. Both clones therefore contain genomic sequences that are contiguous in wild-type DNA, indicating that the RB fragments in the *era1-1* mutant represent the ends of a single-site T-DNA insertion. pSC10 was used as a probe to screen an *Arabidopsis* cDNA library, PRL2 λ-ZipLox (ABRC, stock CD4-7); and five positive cDNAs were identified and sequenced. The longest cDNA, pZL51 (1.45 kb), was used to screen Columbia (λ-ZAPII, Stratagene) and Lansberg genomic (λ-FIX, ABRC stock CD4-8) libraries and four positive clones were identified. One clone spanning 6 kb and encompassing the entire pZL51 clone was completely sequenced. A larger genomic insert (14 kb) was used to size deletions in the fast-neutron mutants (*era1-2* and *era1-3*).
12. B. W. Shirley, S. Hanley, H. M. Goodman, *Plant Cell* **4**, 333 (1992).
13. L. E. Goodman, C. M. Perou, A. Fujiyama, F. Tamanoi, *Yeast* **4**, 271 (1988); W-J. Chen, D. A. Anders, J. L. Goldstein, D. W. Russell, M. S. Brown, *Cell* **66**, 327 (1991); Z. Yang, C. L. Cramer, J. C. Watson, *Plant Physiol.* **101**, 667 (1993).
14. W. R. Schafer and J. Rine, *Annu. Rev. Genet.* **30**, 209 (1992).
15. To assay farnesylation, 1 g (fresh weight) of wild-type or mutant flower buds was homogenized in extraction buffer [50 mM Hepes (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 5 mM dithiothreitol (DTT), leupeptin (2 μg/ml) aprotinin (2 μg/ml), and 1 mM phenylmethylsulfonyl fluoride]. The synthetic heptapeptides GGCCAIM (CAIM) and GGCCAIL(CAIL) (*19*) were prepared as described [B. He *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11373 (1991)]. Target peptide sequences were chosen according to activities measured in tobacco culture [S. K. Randall *et al.*, *Plant Cell* **5**, 433 (1993)]. Extracts were clarified at 4°C at 10,000*g* for 10 min and then at 100,000*g* for 30 min. Soluble protein extract (100 μg) was incubated at 30°C for 40 min in 25 μl of reaction buffer [50 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 5 mM DTT, 50 μM peptide, and 0.5 μM [^{3}H]farnesyl diphosphate (FPP) (17.0 Ci/mmol; Amersham)]. Reactions were terminated with EDTA (at a final concentration of 50 mM), spotted onto Silica Gel 60 thin-layer chromatography plates (Millipore), and developed with *n*-propanol and water (7:3 v/v) for 4 to 5 hours. The plates were dried, sprayed with En^{3}Hance (New England Nuclear), and exposed to Kodak X-OMAT AR film at −70°C for 4 days.
16. J. Hancock, H. Paterson, C. Marshall, *Cell* **43**, 133 (1990); S. Leevers, H. F. Paterson, C. J. Marshall, *Nature* **369**, 411 (1994); D. Stokoe, S. G. Macdonald, K. Cadwallader, M. Symons, J. F. Hancock, *Science* **264**, 1463 (1994); P. J. Casey, *Biochem. Soc. Trans.* **23**, 161 (1995).
17. P. C. Sternweis, *Curr. Opin. Cell Biol.* **6**, 198 (1994).
18. J. Inglese, W. J. Koch, M. G. Caron, R. J. Lefkowitz, *Nature* **359**, 147 (1992).
19. 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.
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TECHNICAL COMMENTS

CD2: An Exception to the Immunoglobulin Superfamily Concept?

A central tenet of the immunoglobulin superfamily (IgSF) concept is that the evolutionary success of IgSF domains derives from their ability to form stable protein modules at the cell surface (*1*, *2*), and this is entirely consistent with the high degree of conservation of framework residues in IgSF domains revealed by structural studies (*3*). The recent proposal by Daniel F. Wyss *et al.* (*4*) that some IgSF domains, such as the ligand binding domain of the human T lymphocyte antigen, CD2, "maintain their stable conformation as a result of dynamic interactions between the polypeptide and its attached glycan," is incompatible with the IgSF concept and therefore warrants careful consideration.

CD2 is an integral membrane glycoprotein that has two extracellular IgSF domains N-glycosylated at three sites [reviewed in (*5*)]. The ligand- and antibody-binding properties of a soluble form of CD2 consisting of both IgSF domains are indistinguishable before and after reduction of the N-linked glycans to single N-acetylglucosamine residues by endoglycosidase H treatment (*6*). After complete removal of the glycans with N-glycanase, the protein also binds ligand with wild-type affinity, although the binding of some antibodies is reduced in accord with the reduction in protein solubility observed after complete deglycosylation of CD2 (*6*). These results suggest that the N-linked glycans do not stabilize the folded conformation of two-domain CD2, but may enhance its overall solubility. In contrast, the proteolytic fragment consisting of domain 1 of CD2, on which Wyss *et al.* base much of their analysis, appears to be metastable because N-glycanase treatment of this fragment completely abrogates ligand and antibody binding (*4*, *7*). These differences in the behavior of the single- and two-domain forms of CD2 undermine the biological significance of the effects observed by Wyss *et al.* as there is no counterpart in nature for the single domain form of CD2. It is also relevant that protein stability artifacts have been encountered ever since the earliest attempts were made to produce truncated soluble forms of IgSF molecules by recombinant DNA methods [see, for example, (*8*) and (*9*)]. In the light of these discrepancies and technical uncertainties, it seems inappropriate to conclude that CD2 is a clear exception to the IgSF concept.

These observations do not rule out the possibility that the domain 1 glycan has some other role in CD2 expression and this is clearly implied by the mutational data of Wyss *et al.* The problem with the mutational approach, however, is that it does not necessarily distinguish between the potential effects of glycosylation on (i) protein folding, (ii) post-folding conformational stability, or (iii) protein trafficking to or beyond the cell surface. In view of the conformational stability of deglycosylated two-domain CD2 (*6*, *10*) and the unimpaired trafficking of unglycosylated mutant forms of CD2 to the cell surface (*4*, *7*), it would appear that the domain 1 glycan influences the initial folding of CD2 rather than determining, as proposed by Wyss *et al.*, its post-folding stability. A fluorescence energy transfer study of peptides used as model folding intermediates (*11*), which suggests that N-linked glycans alter the conformational space available to β-turn glycopeptides that are analogous to the glycosylated DE loop of CD2 domain 1, is consistent with this possibility.

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REFERENCES

1. A. F. Williams, *J. Theor. Biol.* **98**, 221 (1982).
2. _____ and A. N. Barclay, *Annu. Rev. Immunol.* **6**, 381 (1988).
3. Y. Harpaz and C. Chothia, *J. Mol. Biol.* **238**, 528 (1994).
4. D. F. Wyss *et al.*, *Science* **269**, 1273 (1995).
5. S. J. Davis and P. A. van der Merwe, *Immunol. Today*