The structure of tobacco ringspot virus: a link in the evolution of icosahedral capsids in the picornavirus superfamily

Veda Chandrasekar^{1,2} and John E Johnson^{1*}

Background: Tobacco ringspot virus (TRSV) is a member of the nepovirus genus of icosahedral RNA plant viruses that cause disease in fruit crops. Nepoviruses, comoviruses and picornaviruses are classified in the picornavirus superfamily. Crystal structures of comoviruses and picornaviruses and the molecular mass of the TRSV subunit (sufficient to accommodate three β -barrel domains) suggested that nepoviruses may represent a link in the evolution of the picornavirus capsids from a T = 3 icosahedral virus. This evolutionary process is thought to involve triplication of the capsid protein gene, to encode a three-domain polyprotein, followed by development of cleavage sites in the interdomain linking regions. Structural studies on TRSV were initiated to determine if the TRSV subunit corresponds to the proposed uncleaved three-domain polyprotein.

Results: The 3.5 Å resolution structure of TRSV shows that the capsid protein consists of three β -barrel domains covalently linked by extended polypeptides. The order of connectivity of the domains in TRSV confirms the proposed connectivity for the precleaved comovirus and picornavirus capsid polyprotein. Structural differences between equivalent domains in TRSV and comoviruses are confined to the external surface loops, interdomain connecting polypeptides and N termini. The three different domains within TRSV and comoviruses are more closely related at the structural level than the three individual domains within picornaviruses.

Conclusions: The structural results confirm the notion of divergent evolution of the capsid polyproteins of nepoviruses, comoviruses and picornaviruses from a common ancestor. A number of residues were found to be conserved among various nepoviruses, some of which stabilize the quaternary structure of the three domains in the TRSV capsid protein subunit. Two conserved regions were identified on the external surface of TRSV, however, mutational studies will be needed to understand their functional significance. Nepoviruses transmitted by the same nematode species do not share regions with similar amino acid composition on the viral surface.

Introduction

High-resolution structures of several icosahedral RNA viruses belonging to eight different families have been determined (e.g. [1]). The capsids of these viruses are composed of 180 subunits and fall into two categories: the T = 3 viruses, in which all the subunits are formed from the same gene product, and the pseudo T = 3, picorna-like viruses, in which there are 60 copies of three different, but structurally similar, subunits. The three different subunits in the latter particle are synthesized as a polyprotein and occupy positions in the surface lattice comparable to those of the single gene product in a T = 3 virus.

Tobacco ringspot virus (TRSV), a small icosahedral RNA plant virus, is a member of the nepovirus genus [2,3]. The TRSV capsid is composed of 60 copies of a single capsid

Addresses: ¹Department of Molecular Biology, MB-31, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA and ²Department of Biological Sciences, Purdue University, 1392 Lilly Hall of Life Science, West Lafayette, IN 47907, USA.

*Corresponding author. E-mail: jackj@noda.scripps.edu

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protein (56,000 Da; 513 amino acids [4,5]). The nepovirus and comovirus genera in the comoviridae family are classified in the picornavirus superfamily. This classification is based on their similarity to animal picornaviruses in the translation of the genomic RNA as a polyprotein, the cleavage of this polyprotein by a virally encoded proteinase, and the sequence similarity among non-structural proteins [6]. Nevertheless, there is no statistically significant sequence similarity between the capsid polyproteins of nepoviruses, comoviruses and picornaviruses. Previous structural studies of comoviruses and picornaviruses have suggested that picorna-like viruses have evolved from T = 3 viruses by triplication of the gene coding a β -barrel domain into a three-domain polyprotein, followed by independent evolution of the β barrels and the development of cleavage sites in the capsid polyprotein [7,8]. The picornavirus capsid polyprotein is cleaved at two sites to yield three subunits, each of which is folded into a β barrel domain. The comovirus polyprotein, on the other hand, is cleaved at only one site to yield two subunits: one composed of two β barrels and the other composed of one β barrel. Given the size of the nepovirus capsid protein, it was hypothesized that its capsid protein may contain three covalently linked β -barrel domains, and that nepoviruses may represent an example of an early stage in the development of picornaviruses [7]. The structure determination of a nepovirus and the structural comparison with comoviruses is, therefore, of evolutionary significance.

In addition to protecting the encapsidated nucleic acid from degradation, the capsid of a virus plays a crucial role in several aspects of the viral life cycle. In the case of nepoviruses, previous studies have indicated the role of the capsid in interactions with the soil-inhabiting nematode (eel worm) vector; these interactions dictate specificity during virus transmission [9,10]. The capsid has also been implicated in the movement of the virus across the plant cell wall through cytoplasmic connections formed by the viral movement protein (and other unknown host factors [11]). Hence, an examination of the surface topography of a nepovirus capsid structure may provide structural insights into these functions.

The TRSV genome is bipartite and consists of two positive-sense, single-stranded RNA molecules: RNA1 $(2.6 \times 10^6 \text{ Da})$ that encodes four non-structural proteins; and RNA2 $(1.2 \times 10^6 \text{ Da})$ that encodes the capsid protein and an upstream movement protein. The two RNA molecules are encapsidated separately in particles of 280 Å diameter [12,13]; both particles are required for infection. The virus particles purified from infected plants sediment as three separate components, depending on the RNA content, and include empty capsids containing no RNA [2]. The crystals used in the present study contain a mixture of all the three components. Here we present the 3.5 Å structure of the first nepovirus, TRSV, and a structural comparison of TRSV with comoviruses. Our structural results support the evolutionary link among the three groups in the picornavirus superfamily and confirm the connectivity of the domains in the nascent polyprotein proposed from the structures of picornaviruses [14,15] and comoviruses [7].

Results and discussion Structure of the whole capsid

The capsid of TRSV consists of a protein shell with an average thickness of 40 Å (Figure 1a). The outer and inner surfaces of the capsid display considerable variation in the radial dimension resulting in a non-spherical density distribution. The most prominent features on the outer surface are the pronounced protrusions near the icosahedral fivefold axes. There is a minor protrusion at each

icosahedral threefold axis and a depression near each icosahedral twofold axis, where the capsid has a minimum thickness of 14 Å. The inner and outer radial dimensions along the icosahedral symmetry axes (Figure 1a) are in agreement with the cryo-electron microscopy (cryo-EM) reconstruction of TRSV virus-like particles (VLPs) that result from expressing the capsid protein gene in a baculovirus system [16]. A comparison of the dimensions of TRSV with the comovirus cowpea mosaic virus (CPMV) [7,17] demonstrates that the overall capsid shape of the two viruses is similar.

Quality of the electron-density map

The overall quality of the electron-density map at 3.5 Å resolution is very good (Figure 1b) in spite of the lack of completeness in the diffraction data. The density corresponding to the polypeptide backbone is continuous at a level of 2σ above the background. Most of the amino acid sidechains have well-defined density, allowing the assignment of the known sequence for the TRSV capsid protein without ambiguity. All 513 residues present in the capsid protein were modeled as one continuous chain.

Structure of the capsid protein

The TRSV capsid is composed of 60 copies of a single capsid protein with domains arranged in a pseudo T = 3 icosahedral lattice [18] (Figure 2a). The capsid protein is folded into three trapezoid-shaped β -barrel domains (designated as C, B and A from the N to the C terminus) that are covalently linked together as 'beads on a string'. The B and C domains lie side-by-side with their narrow ends packed together around the virus threefold axes (Figure 2a). The β barrels in these domains lie with the strands of the β sheets tangential to the capsid surface. The prominent protrusion along the fivefold axes is formed by the tilted packing of the β barrels in the strands run roughly parallel to the nearby fivefold axis (Figure 2a).

The connected domains in the TRSV capsid protein (Figures 2b and 2c) agree with the connectivity proposed for the precleaved comovirus and picornavirus capsid polyprotein protomer [7,14,15]. The C domain (containing 179 amino acids) forms the N-terminal third of the capsid protein, the B domain contains the central third of 170 amino acids and the A domain (containing 164 amino acids) constitutes the C-terminal third of the protein. The junction point within the two extended polypeptides linking the domains was assigned based on the structure of the comovirus beanpod mottle virus (BPMV) [17].

The tertiary structure of the wedge-shaped C, B and A domains in TRSV (Figure 2c) is a variation of the canonical eight-stranded antiparallel β -sandwich fold, where the eight strands βB to βI form the BIDG and the CHEF β sheets [19,20]. The twist of the β strands constituting the



The quality of the 3.5 Å electron-density map at 2σ contour level. (a) A thin slab of the electron-density map viewed down the crystallographic twofold axis and containing the central section of the TRSV particle. The inner and outer radial dimensions along the icosahedral symmetry

axes are indicated beneath the axes labels. (b) Density corresponding to the sidechains of the residues Asn356, Trp357, Phe358, Thr359, Leu360 and Thr361 forming the β B strand in the A domain.

barrels in the three domains vary. As in comoviruses, the strands in the B domain have the largest twist relative to the other domains. In general, the loops connecting the strands of the β barrel in the three domains are short, but some are of sufficient length to adopt a secondary structure. Inserts of this type in TRSV are also present in the corresponding positions in comoviruses.

The C domain in TRSV has two helical insertions: the $\beta C-\beta D$ insertion containing the $\alpha A'$ and αA helices; and the βE - βF insertion containing the αB helix. The αC helix present in comoviruses between the βG and βH strands is absent in TRSV. The largest insertion in the B domain occurs between the βC and βD strands and includes two antiparallel β strands, $\beta C'$ and $\beta C''$, and the αA helix. The $\beta E - \beta F$ insertion containing the αB helix is also present in this domain. The only insertion in the A domain is between the β C and β D strands; the insertion contains an extra β strand $\beta C''$ and the αA helix. Unlike comoviruses, where the strands $\beta C'$ and $\beta C''$ lie on top of the canonical β barrel leading to an unusual ten-stranded structure, in TRSV there is no $\beta C'$. The $\beta C''$ strand, however, does lie on top of the CHEF wall. The short N-terminal tail in the C domain, containing only non-basic sidechains, extends on the inner surface of the capsid to the B domain of the threefoldrelated capsid protein. The C terminus of the capsid protein present in the A domain forms an extra strand, $\beta I'$, and extends toward the outer surface of the virus. The similarity between the C α traces of the three domains in TRSV (Figure 3) support the hypothesis that the three β barrels in the nepovirus subunit have evolved from a T = 3 virus as a result of gene triplication.

Interdomain connecting polypeptides

The extended polypeptide of 25 residues that connects the C and the B domains (Figure 4a), starts from the end of the β I strand in the C domain. The polypeptide runs roughly perpendicular to the β strands of the C domain on the inner surface of the capsid until it reaches the A domain, where it makes a sharp upwards turn and runs alongside the end of the β I strand in the A domain. Finally, the linking peptide makes another sharp downwards turn and reaches the β B strand in the B domain. The equivalent C–B domain-connecting polypeptide in comoviruses contains 14 residues and has a very different conformation (Figure 4b). This polypeptide in BPMV defines one side of a shallow pocket between the B and C domains that serves as the binding site for ordered RNA [7].

The B-A domain-connecting polypeptide of 15 residues, starts from the end of the β I strand in the B domain. This polypeptide runs perpendicular to the strands in the B domain on the inner surface of the capsid and then makes a turn at the residue Pro347 and ends at the β B strand in the A domain. The equivalent B-A domainconnecting polypeptide in comoviruses is formed by the C-terminal tail of the large subunit and the N-terminal tail of the small subunit, which arise as a result of







Structure of the TRSV capsid, capsid protein and the three domains in the capsid protein. (a) A CPK model of the pseudo T = 3 TRSV capsid showing the prominent surface features. The C and B domains (in green and red, respectively) are clustered around the threefold axes, while the A domains (cyan) are clustered around the fivefold axes. (b) An enlarged view of one of the 60 copies of the capsid protein in the TRSV capsid represented as a CPK model. (c) A cartoon representation of the tertiary structure of the C, B and A domains in the capsid protein. The secondary structure features in the three domains, the two domain-linking polypeptides and the N and C termini of the capsid protein are indicated. (Figure 2c was generated using MOLSCRIPT [40] modified by Robert Esnouf.)

cleavage of the capsid polyprotein at a specific site in this loop by the virally encoded proteinase. The precleaved B–A domain-connecting polypeptide contains 30 to 36 residues in other comoviruses and the post cleaved N and C termini are separated from each other in BPMV and CPMV. While the new C termini of the B domains are in similar positions in BPMV and CPMV, the new N termini of the A domains have very different structures in these two comoviruses.

Quaternary interactions within the capsid protein

The quaternary interactions occurring within the TRSV subunit are defined by contacts at the A–B₅, C–B₅ and A–C interfaces (Figure 5a). The interactions at the C–B₅ and the A–B₅ interfaces are significantly more extensive than at the C–A interface. This suggests that the three domains C, B₅ and A in the TRSV capsid protein associate with each other successively as they are synthesized. Hydrophobic interactions play a dominant role in establishing the

Stereo view C α traces of the **(a)** C, **(b)** B and **(c)** A β -barrel domains in TRSV. The traces are all shown in the same orientation and every tenth residue is labeled.



quaternary organization of these domains in both TRSV and CPMV. There is no intertwining of the N and C termini in

nepoviruses and comoviruses, while this is an important stabilizing interaction in picornaviruses [14,15,21,22].





Interdomain linking polypeptides in TRSV. (a) Stereo view of the C α trace (white) of the capsid protein from inside the capsid with the electron density for the two domain-linking polypeptides. The pink density shows the link between the C and B domains and the brown density shows the link between the B and A domains. (b) An enlarged view of (a) showing the difference in the conformation of the C-B domain-connecting polypeptides in TRSV (yellow) and BPMV (green).

Quaternary interactions between the capsid proteins

Quaternary interactions occur between pairs of capsid proteins related by icosahedral fivefold, threefold and twofold axes in the pseudo T = 3 capsid of TRSV.

Quaternary interactions at the fivefold contact consist of A–A₅, A–B and B–C domain interfaces (Figure 5b). The interactions at the A–A₅ and A–B interfaces are much more extensive than those at the B–C interface. Unlike comoviruses, where polar interactions play a dominant role in the association of the capsid proteins into a pentamer, hydrophobic interactions are more important in TRSV.

The only interdomain interface that defines the threefold contact between subunits in TRSV is $C-B_2$ (Figure 5c). Hydrophobic interactions and the N terminus play a prominent role in the association of the capsid proteins

around the threefold axes. In contrast the N-terminal tail is not involved in this contact in CPMV. Protein–RNA interactions are involved in this contact in BPMV.

The twofold-related subunits contact each other only at the C-C₂ interface (Figure 5d). The interactions at this interface in TRSV are minimal. The N terminus of the C domain in comoviruses extends to the RNA-binding pocket in the twofold-related C₂ domain. The pseudo T = 3 nature of the TRSV capsid suggests comparing the A-B₅ contact with the C-C₂ contact as they are quasiequivalent in the capsids formed by a single subunit type. The quasi or pseudo-equivalence breaks down dramatically in TRSV because the chemical interfaces are different at the two contacts even though similar structural elements are adjacent (Figure 5d,e). Interactions at the A-B₅ contact.

Quaternary interactions between the TRSV capsid proteins. The C, B and A domains in the capsid are shown in green, red and cyan respectively. (a) AT = 3 icosahedral surface lattice represented as a rhombic triacontahedron, viewed approximately down an icosahedral twofold axis normal to the plane of the paper. The figure indicates the domains involved in fivefold, threefold and twofold contacts between capsid proteins. Closed pentagons, triangles and the closed oval symbolize the icosahedral fivefold, threefold and twofold axes, respectively. Open triangles and the open oval represent the quasi-threefold and twofold axes, respectively. The trapezoids labeled C, B₅ and A constitute three β -barrel domains that form one polypeptide chain going from the N to the C terminus. (b) Fivefold contact between capsid proteins viewed in the same orientation as (a). The three domain interfaces at this contact bury nearly 4600 Å² of the accessible surface area. (c) Hexamer of the B and C domains around the icosahedral threefold axes viewed in the same orientation as (a). Nearly 2570 Å² of the accessible surface area becomes buried at this threefold contact. (d) The icosahedral twofold contact at the C-C₂ domain interface (viewed down the icosahedral twofold axes) versus (e) the quasi-twofold contact at the A-B5 domain interface (viewed approximately down the guasi-twofold axes). Only 606 Å² of the accessible surface area is buried at the $C-C_2$ interface compared to 2120 Å² at the A-B₅ interface.



The fivefold contacts between the capsid polyproteins are the strongest in the assembled capsid of TRSV and CPMV. This observation suggests that the TRSV capsid proteins assemble into pentamers rather than dimers or trimers, although experimental evidence for this has not been reported for nepoviruses or comoviruses. An assembly intermediate of picornaviruses has been identified which sediments at 14 S and is composed of a pentamer of three β sandwich subunits, VP1, VP2 and VP3, which correspond to A, C and B in TRSV [23,24].

Sequence alignment of nepoviruses

A multiple sequence alignment of the capsid proteins of 11 nepoviruses and five comoviruses [25] suggested that the two genera may share a common structure despite the low sequence identity within the nepovirus genus and between the two genera of viruses. A structural prediction for nepoviruses was attempted on the basis of the sequence alignment and the known comovirus structures. The predictions of the C and B domain structures were of reasonable quality when compared with the TRSV structure reported here. Significant differences between the predicted and actual structures were found in the A domain. A major readjustment of the structure prediction of the nepovirus cherry leaf roll virus (CLRV) was required. In Figure 6 a new sequence alignment of all the nepoviruses is shown with the reported TRSV structure as the reference.

Figure	6
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				1	βB	I6	6C αA'-	- αA"-	β ⊡' -
TR	sv			avtvvpdp	tcCatlsfkV	PkDakkGkhL	qtfDIrgaIm	dvaalhsaeW	cakGiVnptf
 Тс	RSV			aaswaeat	eaAvlakvtC	AkDakgGtlL	htlDTikeCk	sanllrvkeW	araGfLhakl
RR	SV			avevdpl	hllwvesvdV	PkDtlaGtlL	ariDVrakAa	ifnsavwrow	vrdGcLknki
ΨE	RV-FD			Tdp10fp	dfacgetijL	PaTeacGeiL	akiDLiglTk	ntntrycseW	lmdGvVscml
75 775	DV-C			aag	sylfgetieL	PartnGtyr	awfNTfdkTa	otntkycskW	leaCyVsqnl
10				agg	oflibtid	PtlvtoCoul	avinitukiq	dakamuauaw	maaCuWnknl
GC	.191 V			agg	elalincial	PLAVLEGQVL	aKIDIIKKIQ	daksiivevqw	ingaGyviikiii
GF	.ГЛ				glAgrgviyl	PKDCqaNryL	gtiNiramis	aikgvqyekw	itaGIVmpti
Ar	ſΜV				glAgrgsvqV	PkDcqaGiyL	ktlDLrdmVs	gisgiqyekW	itaGiVmpnf
SL	RSV	gfhedlvpaa	sggteaiffs	pksipvpgsa	kfVgshpfsF	PiNsnvGttV	yslPListSl	kdtewgrY	yr-SyTfmrf
CL	JRV			sritpgm	hwSavtpfkC	AaEaaeNtiL	arwSLrsiIs	esgtdawtkW	qreLpttf
BL	JMV			sgliadt	siAhvvqgwV	PkDatkGrvL	eaiNLredIa	tsdnlvkye₩	lakGlIhpdl
		βD		-βE		αB	F	-βG-	βg '
TR	lsv	TVrmha	prNaFaGLsI	aCtFDDYkRi	dlpalgn-ec	PpsemfelPt	kVfmLKDadv	heWqFNyg-e	LTGhgLcnwa
Tc	RSV	RLrcfi	ptNiFcGHsM	mCsLDAFgRy	dsnvlga-sf	PvklasllPt	eVisLADgpv	vtWtFDig-r	LCGhgLyyse
RR	sv	KMrita	atScFsGIvL	gAcFDAYrRi	paatktg-i-	TaslvtglPn	tVwaTRDtse	veWdIDla-a	VCGhtFfale
ΨP	RV-ED	RAvshl	apNaEsGISI	wYiFDAYaKi	padistt-ie	LemakclsP-	hVgtLRDatt	ssWiTDfh-k	MCGatLnfsa
 TE	RV-S	TAighl	apNaEsGIaI	wViFDAVcKi	padyttt-fe	LemarsfdP-	hVovLRDvst	stWyIDfh-k	ICCatInfsa
CC	MT7	TFichl	apforectual	wViFDAVaKi	pgdvttte_le	LeiarsfdP-	hVhvLPDckt	syWtIDfh-k	ICCgcLnfcg
GC	יניג. ז די 7	VIII	apsqrcGvar noNoEtCI tw	WIIPDAIGRI	taritag ad	Durit la uDh	WI ibuKUskt	focolDura o	ICChaMwfkg
Gr			panartGLtw	VMSFDAIIRI	theight og	Poytis-vPh	whillinkig-t	tagdi Dug a	LCGHAMWIKS
AL	MV	KVVILY	panartGItw	VMSFDATHRI	llSISIL-dS	Paytis-VPn	WLINHKNG-L	LiscalDyg-e	LCGHamwiga
SL	IRSV	KPtvrlis	sapiQakGIL	wLcyDPCeTI	аку	PsreralmLq	gvwiMPGr	ndSvTLtide	LATpsGysim
CL	JRV	LVtgtiam	svNiMaGTtL	gLvCDVFnR-	sklldtf	PstlgqnmPq	rVfpLSNple	rnFsFSmg-e	LTGhtMhpqa
BL	JMV	KLrmtv	gqNpFvGIsI	gIcCDYFgRl	skyyegdtal	PievcnqlPn	fVcpISEksv	feFdLDms	LAGynLfqts
	133	β – βι	∃	βI	C-B lir	king polype	ptide	- βB	βC
TR	lsv	nvAtgPtLyF	fVAStNOVtm	aADWgciVtm	hvdmgpvidr	felnPtMtwP	iqlqDtfaId	rvyeAkeIkL	dGstsmlsis
ТС	RSV	gaYarPkTvF	1VTSdNDVpa	eADWaft.Yal	lfedhtfsns	fgavPfTt1P	-hifNrldTa	vwrgPteIdL	tStpapnavr
RR	SV	dt FayMdFl T	vVLBaNETta	vADWsivVsf	hydwtaesml	atliPtFywP	nkntDislLk	evwaPvrFtL	dGteakesfa
TCI-		ngEckPtLyW	y Mignered wWASoFOLar	e AFTkfrLef	vataerlura	lconP-LtvP	ikpaHledLd	lvlkSggTaT	-Ctytmtkyro
		pgrcki chyv	i AAStEOLar	amAmkfriof	yacgerivig	loogD LovD	ioprultdin	lmlaDkgTaV	Cturnitfo
TE	KV-S	QQICVPKIWV	IAAStrular	STATKLILEL	yrigekivig	LaeqP-LsyP	Tearnitun	InitaPkqiav	-Guyamitup
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GC	CMV	rgFskPtLwV	IAAStAQLpw	sAQVtyrLea	laqgdeiahg	latrSiVtyP	isleHlkdIe	imlpPrqMaI	-Gnagsinfp
GC GF	'LV	rgFskPtLwV ttFesPrLhF	1AAStAQLpw tCLTgNNKel	sAQVtyrLea aADWqavVel	laqgdeiahg yaeleeatsf	latrSiVtyP lg-kPtLvfD	isleHlkdIe pgvfNgkfQf	imlpPrqMaI ltcpPifFdL	-Gnagsinfp tAvtalrsag
GC GF Ar	'LV 'LV MV	rgFskPtLwV ttFesPrLhF ttFesPkLhF	1AAStAQLpw tCLTgNNKel tCLTgNNKel	sAQVtyrLea aADWqavVel aADWefvVel	laqgdeiahg yaeleeatsf yaefeaaksf	latrSiVtyP lg-kPtLvfD lg-kPnFiyS	isleHlkdIe pgvfNgkfQf ldafNgslKf	imlpPrqMaI ltcpPifFdL ltipPleYdL	-Gnagsinfp tAvtalrsag sAtsayksvs
GC GF Ar SL	'MV 'LV MV .RSV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp	imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka
GC GF Ar SL CL	MV LV MV RSV RV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin
GC GF Ar SL CL BL	MV LV MV RSV RV MV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV	1AAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP</pre>	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd</pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren
GC GF Ar SL CL BL	:MV 'LV :MV .RSV .RV .MV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP</pre>	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd</pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren
GC GF Ar SL CL BL	:MV 'LV :MV .RSV .RV .MV 213	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV β βC'	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats βD	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP</pre>	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd βΕ </pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren (B βF
GC GF SL CL BL	:MV 'LV :MV .RSV .RV .MV 213 2SV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV ββC' ynfGgpvkh-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa -&C' - <u>s</u> -kkhai	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats βD lGwSGtIsgs	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc</pre>	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd βE TasFvIfp-W</pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL -0 ec-eapptlr</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren (B βF <u>QVlwgPHqiM</u>
GC GF Ar SL CL BL TR TC	:MV 'LV :RV .RSV .RV .MV 213 2SV oRSV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvL1V ββC' ynfGgpvkh- llfGlstvi-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats βD lGwSGtIsgs gGsNGtLhgr	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP / VksvSS1-Fc IkkiGTa-Lt</pre>	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd βE TasFvIfp-W TcsLlLslrH</pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL -0 ec-eapptlr kdasltle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren αB βF <u>QVlwgPHqiM</u> TAyqrPHyiL
GC GF Ar SL CL BL TR TC RR	:MV 'LV :RSV .RV .RV .MV 213 2SV 2SV 2SV 2SV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV ββC' ynfGgpvkh- llfGlstvi- impGtailh-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats βD lGwSGtIsgs qGsNGtLhgr rSwTGkVrms	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IgevSSi-Fl	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLLslrH TgtYMVgvsW	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren (B βF <u>QVlwgPHqiM</u> TAyqrPHyiL DIvtrKHwiV
GC GF Ar SL CL BL TR TC RR TC RR TE	:MV LV MV RSV RV MV 213 RSV RSV RSV RSV RSV	rgFskPtLwV ttFesPthF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 9 βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa - <u>s</u> -kkhai -sgnmstl -gqqivr -aakrgav	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf SvaagvLShf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGdIifs	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv</pre>	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd I βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaciPHydV
GC GF Ar SL CL BL TR TC RR TC TE	MV LV MV RSV RV MV 213 SV RV RV SV RV ED RV-ED RV-S	rgFskPtLwV ttFesPthF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 9 βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAklgs-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa - <u>s</u> -kkhai -sgnmstl -gqqivr -aakrqay -tsgrtav	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev r- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGLifs lGvGGLIfs	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP / VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv</pre>	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd I βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptIr kdasltle ngvtndla gtiptte gtvpetd</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV OLacmPHvdV
GC GF Ar SL CL BL TR TC RR TC RR TE GC	MV LV RV RSV MV 213 SV SV SV SV SV SV SV SV SV SRV-ED SRV-S MV	rgFskPtLwV ttFesPtLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV sβ fGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvggks-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa - <u>s</u> -kkhai -sgnmstl -gqqivr -aakrqay -tsgrtay	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev '- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-FV VrttSSa-Fv UgctSSa-Fv	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL <u>ec-eapptlr</u> kdasltle ngvtndla gtvpetd gtvpetd</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren 2B βF <u>QVlwgPHqiM</u> TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV OLIscmPHvdV
GC GF Ar SL CL BL TR TC RR TC RR TE GE	MV LV MV RSV RSV MV 213 SV SV SV SV SV SV SV SV SV SV SV SV SV	rgFskPtLwV ttFesPtLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAaklqs- lsfAvqqks- ltfGuymy-	1AAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa <u>-g</u> -kkhai -gqqivr -aakrqay -tsgrtay -ssgriay	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NangalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats βD lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGGdIfs lGvGGtIhfv lGiGGtIhfk	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv VhctSSa-Fv VhicSSa-Fv</pre>	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd I βE TasFvIfp-W TcsLlslrH TgtYmVgvsW ScSLRSValW TskLrIal-W TarLrVal-W	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL <u>ec-eapptlr</u> kdasltle ngvtndla gtiptte gtvpetd gdtitle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren 2B βF <u>QVlwgPHqiM</u> TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdC BL.frvGrvU
GC GF Ar SL CL BL TR TC RR TE GC GC	MV 'LV MV RSV RV MV 213 SV PRV SV PRV-ED RV-ED RV-S MV 'LV MV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 βC' ynf <u>Ggpvkh</u> - llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpwd-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yVLNtNDVac gyLDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev '- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf NlnstlVScv	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats βD lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtIhfv	<pre>latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv IqctSSa-Fv VhicAPi-Fy VhicAPi-Ty</pre>	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd [βE] TasFvIfp-W TcsLlLslrH TgtYmVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gtttle ngtttle ngtttle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren ΔB βF <u>QVlwgPHqiM</u> TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdC ELfkyPGvyV
GC GF Ar SL CL BL TR TC RR TE GC GF	MV LV RV RV MV 213 SV 253 SV 254 SV SV SV SV SV SV SV SV SV SV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV BβC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd-	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats βD lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfk lGmGGtVrgr lGiGGiVkgk	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYmVgvsW ScSLRSValW TskLrIal-W TarLrVal-W GivLrVvseW GrtVaM2 - E	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGcyI DLvrCctV
GC GF Ar SL CL BL TR TC RR TC GC GF Ar	MV LV MV RSV RV MV 213 SV RSV RSV SV SV SV SV SV SV SV SV SV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV β βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp	LAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIfs lGvGGtIhfv lGiGGtIhfk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv IqctSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL o ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gttile ngttmdwn ngvtnnwn andsysgwes</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren (B βF QV]wgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV
GC GF Ar SL CL BL TR TC RR TC GC GF Ar SL CL	MV LW RV RV RV MV 213 SV RV RV SV SV SV SV SV SV SV SV SV S	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV ββC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaept	LAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtNfkk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd I βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GjvLrVvseW GgtVeMgF QvdIaLam-W	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvLL
GC GF Ar SL CL BL TR TC RR TC RR TE GC GF Ar SL CL BL	MV LV RV RSV RV MV 213 SV SV SV SV SV SV SV SV SV SV SV SV SV	$rgFskPtLWV$ $ttFesPtLhF$ $rsDhnGaFkV$ $taFedVqFiL$ $kgFadPvL1V$ $g \beta C'$ $ynfGgpvkh-$ $llfGlstvi-$ $imgGtailh-$ $vslAmrvds-$ $vslAaklqs-$ $lsfAvqqks-$ $ltIGqvpmv-$ $llGqtlvd-$ $lvvPlnpllp$ $vnlGfaeptt$ $h-iGinfgsa$	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtNfk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd $I\beta E $ TasFvIfp-W TcsLlslrH TgtYmVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GivLrVvseW GgtVeMg-F QvdIaLam-W SceMfLilqP	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ogtptlr kdasltle ngvtndla gtiptte gtvpetd gttitle ngttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI
GC GF Ar SL CL BL TR TR TR TR TR GC GF Ar SL CL BL	MV LV MV RSV RV MV 213 SV SV SV SV SV SV SV SV SV SV SV SV SV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV $\beta \beta C'$ ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa	LAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtISg qGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFVIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W SivLrVvseW GivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ogtpfte ngvtndla gtiptte gtvpetd gtitle ngttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI
GC GF Ar SL CL BL TC RF TC RF TE GC GF Ar SL CL BL	MV LW RV RV RV AV 213 RV AV RV 213 RV AV RV AV RV AV AV AV AV AV AV AV AV AV A	rgFskPtLwV ttFesPthF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV β βC' ynfGgpvkh- llfGlstvi- imgGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaeptt h-iGinfgsa	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGLlhfv lGiGGtIhfk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSsi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GjvLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptIr kdasltle ngvtndla gtiptte gtiptte ngtiptte ngtimdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLagmPHvdV QLagmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI
GC GF Ar SL CL BL TC RF TC RF TC GF GF Ar SL CL BL	MV 'LV 'RV RV RV 213 RV MV RV RV SV RV-ED RV-S MV 'LV MV RV RV RV 285 SV SV SV SV SV SV SV SV SV SV	rgFskPtLwV ttFesPthF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV gnf <u>Ggpvkh-</u> llf <u>G</u> lstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltl <u>G</u> qvmv- lll <u>G</u> qtlvd- lvvPlnpllp vnl <u>G</u> faeprt h-i <u>G</u> infgsa 5 -β <u>G</u> - h-g <u>D</u> gqFeIa	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev r- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGLIhfv lGiGGtIhfk lGiGGtIhfk lGiGGtVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH- LgI <u>lpL</u>	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhicAPi-Fy VhicAPi-Fy VhicASg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVaeW GivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte gtiptte ngtitle ngtitle ngtitle ngtitle ngttmdwn andsysgwes ndnadgvefr dktahnle tWrpds</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren @ βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHv1L QAlr1PGcrI qvhppmfs-s
GC GF Ar SL CL BL TC RR TC RR TC GF Ar SL CL BL TR TC	MV LW RV RV RV 213 RV RV RV RV LV MV RV RV RV 285 MV LV RV RV RV RV RV RV RV RV RV R	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 β C' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa 5 $ -\beta$ G- h-gDqqFeIa adqQqaFsLp	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev r- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagLShf SyaagLShf SyaagLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGLihfv lGiGGtIhfk lGwGGtIhfv lGiGGtVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH- LgIlpL LgIa	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhocSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gtitle ngvtndwn andsysgwes ndnadgvefr dktahnle tWrpds hIel-v</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLsqmPHvdC QLsqmPHvdC QLsfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s egvprtia
GC GF Ar SL CL BL TR TC RR TC GF Ar SL CL BL TR TC RR	MV 'LV MV RSV RV MV 213 SV RV MV 213 SV RV RV RV RV MV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 SV 285 285 285 285 285 285 285 285	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 β C' ynf <u>Ggpvkh</u> - llf <u>G</u> lstvi- imp <u>G</u> tailh- vslAmrvds- vslAaklqs- lsfAvqqks- lt <u>I</u> <u>G</u> qtpw- lll <u>G</u> qtvd- lvvPlnpllp vnl <u>G</u> faeprt h- <u>i</u> <u>G</u> i <u>G</u> h- <u>g</u> <u>D</u> <u>G</u> <u>F</u> <u>I</u> k-sNeiFeVd	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yVLNtNDVac yUIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev αA SysravMSrn NanqalLRff TfprvvAAhf SyaaglLShf SyaaglLShf SyaaglLShf NInstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r ftgqAngk	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtIhfk lGmGGtVrgr lGiGGiVkgk rYwKGLLvle iSyGGtIhgr qSvGGiLhgt βH LgIlpL LeIfaI pfIiVhkL	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhocSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIh	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gtitle ngttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle tWrpds hIel-v gLtg-vyknp</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren 2B βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLagmPHvdV QLfxyPGcyI DAyryFGstV ELikiPHv1L QAlr1PGcrI qvhppmfs-s egvprtia tlhsgdrs
GC GF Ar SL CL BL TR TC RR TE GC GF Ar SL CL BL TR TC RR TC RR TC TC RR TC TC TC TC TC TC TC TC TC TC TC TC TC	MV LW RV RV RV MV 213 RV MV 213 RV RV RV RV RV RV RV RV RV RV	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV $\beta_{\rm GC'}$ ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa $\delta_{\rm GC'}$ h-gDqqFeIa adgQaFsLp k-sNeiFeVd t-lDakAtLq	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIfs lGvGGtIhfv lGiGGtNfk lGiGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH LgIlpL FJIatL	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv cAPlAPetme	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFVIfp-W TcsLLslrH TgtYmVgvSW ScSLRSValw TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI GsveFiVhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIh	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -G-fpstin -Grss-kren CB βF QV]wgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s egyprtia tlhsgdrs ahlcre
GC GF Ar SL CL BL TR TC RR TE GC GF Ar SL CL BL TR TC RR TC RR TE TE	MV LWV RV RV RV MV 213 RV MV RV RV RV MV 285 SV RV RV 285 SV RV RV RV SV RV RV RV RV RV RV RV RV RV R	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa 5 -βG- h-gDgqFeIa adgQgFsLp k-sNeiFeVd t-lDakAtLq e-vNvdAsLq	IAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa 	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf SyaaglLShf SyaaglLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r ftgqAngk fgdGta- fgnsGsa-	<pre>laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats</pre>	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv gGIvGPkdsv cAPlAPetme cAPmAPetve	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI GsyeFiVhIn AkyqFmCyFd GtfgFmIhIh TgfYyIhIh	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -G-fpstin -Grss-kren @ βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s egvprtia tlhsgd-rs -ahlc-re
GC GF Ar SL CL BL TR TC RTE GC GF Ar SL CL BL TR TC RTE TC RTE TC RTE TC RTE TC CL GF TC CL TR TC CL TR TC CL TR TC CL TR TC TC RTC TC RTC TC RTC TC RTC RTC R	MV 'LV MV RSV RV MV 213 SV RSV SV RSV SV RSV MV 285 MV 285 SV RSV SV RSV SV RSV SV RSV SV RSV SV RSV R	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV ββC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa δ -βG- h-gDgqFeIa adgQgaFsLp k-sNeiFeVd t-lDakAtLq e-vNvdAsLq d-vDvvSsLk	lAAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa $ -\beta C'$ \underline{s} -kkhai \underline{s} gnmstl \underline{g} qqivr \underline{a} akrqay \underline{t} sgrtay \underline{g} tkvy \underline{g} fdgaqwyp \underline{v} tgyapit rvvsgtntfy $\beta G'$ IktRlhsaat IstP-haats LycPygenpt IqsPffatan IqsPffstan	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev y- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r ftgqAngk fgdGta- fgdsG-ar	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGwSGtIsgs rSwTGkVrms lGvGGdIifs lGvGGtIhfv lGiGGtNfk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH LgIpL EJTaTL FyIatL FyVstL	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv GIVGPkdsv cAPlAPetme sSPmAPetme	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd I βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIn TgsYyIqIk SkleYyIqII	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -G-fpstin -Grss-kren QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s egvprtia tlhsgd-rs -ahlc-re pglc-re
GC GF Ar SL CL BL TR TC RE TE GC GF Ar SL CL BL TR TC RE TC RE TC RE TC RE TC C GF GF Ar	MV LW RV RV RV RV MV 213 SV RV SV SV SV SV SV SV SV SV SV S	rgFskPtLwV ttFesPrLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV β βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa δ -βG- h-gDgqFeIa adgQgaFsLp k-sNeiFeVd t-lDakAtLq e-vNvdAsLq d-vDvvSsLk ee-DgsFeVk	lAAStAQLpw tCLTgNNKel tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDvac yIIDtNSLpa $ -\beta C'$ $-\underline{s}-kk$ hai -gqqivr -aakrqay -tsgrtay -gtkvy -gtkvy -gfdgaqwyp -vltgyapit rvvsgtntfy $\beta G'$ IktRlhsaat IstP-haats LycPygenpt IqsPffatan IqsPffstan IqsPfyatan	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev '- αA SysravMSrn NanqalLRff TfprvvAAhf SyaagvLShf SyaaglLShf SyaaglLShf NInstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r ftgqAngk fgdsG-ar rllagqSq-r	<pre>laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats</pre>	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSSi-F1 VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv cAPlAPetme cAPmAPetve sSPmAPetme aGPiAPsget	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd TasFVIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W SivLrVvseW GjvLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIh TgfqYyIhIh	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gtiptte ngttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle tWrpds flean gIdad gIdad elvrd</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren QUlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdV QLfkyPGcyI DAyryFGstV ELikiPHv1L QAlrlPGcrI qvhppmfs-s egvprtia tlhsgdrs aplcre ppmcrq dlslpsfe
GC GF Ar SL CL BL TC R R T TC R R T T C GF Ar SL CL BL TR TC R R T E GC GF Ar SL CL BL TR TC R R T C R TC R TC R TC R R TC R TC R R TC TC TC R TC R TC R TC R TC R TC TC TC TC TC TC T T T T	MV LW RV RV RV RV MV 213 SV RV RV RV RV ED RV-S MV RV RV RV RV RV RV RV RV RV R	rgFskPtLwV ttFesPrLhF ttFesPrLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV β βC' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa δ -βG- h-gDgqFeIa adgQgaFsLp k-sNeiFeVd t-lDakAtLq e-vNvAsLq d-vDvvSsLk ee-DgsFeVk	lAAStAQLpw tCLTgNNKel tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa $\[-\beta C' \\ -\underline{s} - kk - hai \\ -gqqivr \\ -aakr - qay \\ -tsgr - tay \\ -ssgr - iay \\ -gttkvy \\ -gttkvy \\ -gfdgaqwyp \\ -vltgyapit \\ rvvsgtntfy \\ \[\beta G' \\ IktRlhsaat \\ IstP-haats \\ LycPygenpt \\ IqsPffstan \\ IqsPffstan \\ IqsPfyatan \\ IrsPyhrtpa \]$	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev y- αA SysravMSrn NanqalLRff TfprvvAAhf SyaaglLShf SyaaglLShf SyaaglLShf NInstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledM1-r ftgqAngk fgdGta- fgdsG-ar rllagqSq-r rlidaqSass	<pre>laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats</pre>	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSS1-Fc IkkiGTa-Lt IqevSsi-F1 VhstASt-Fv VhtcAPi-Fy VhrcSPc-Ty VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv cAPlAPetme cAPmAPetve sSPmAPetme aGPiAPsget	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd TasFvIfp-W TcsLlslrH TgtYmVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIn SkleYyIqII AkpUvQid AkmpVvVqIe	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptlr kdasltle ngvtndla gtiptte ngtiptte ngtiptte ngtiptte ngtiptte ngtimdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle tWrpds flean gIdad eIvrd</pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLagmPHvdV QLagmPHvdV QLagmPHvdV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s egvprtia tlhsgd-rs -ahlc-re -ppdc-re dlslpsfe dlsvpsfp
GC GF Ar SL CL BL TC RR TC RR TE GC GF Ar SL CL BL TR CR E E GC GF Ar SL CL BL TC RR TC RR TC R RE TC R RE TC R TC R RE TC R R TC R SL CL SL SL CL SL SL CL SL CL SL SL CL SL SL CL SL SL CL SL SL SL CL SL SL SL CL SL SL SL CL SL SL SL SL SL SL SL SL SL SL SL SL SL	MV LIV RV RV RV MV 213 RV MV 213 RV RV RV RV RV RV RV RV RV RV	rgFskPtLwV ttFesPrLhF ttFesPrLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV 3 β C' ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa 5 $ -\beta G- $ h-gDqqFeIa adgQgaFsLp k-sNeiFeVd t-lDakAtLq e-VNvAsLq d-vDvvSsL ee-DgsFaIe vdIRahRlLr	<pre>IAAStAQLpw tCLTgNNKel tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yVLNtNDVac yUIDtNSLpa</pre>	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev y- αA SysravMSrn NanqalLRff TfprvvAAhf SyaaglLShf SyaaglLShf SyaaglLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledM1-r ftgqAngk fgddGta- fgdsG-ar rllagqSq-r rlidaqSasf	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats lGwSGtIsgs gGsNGtLhgr rSwTGkVrms lGvGGLIhfx lGvGGtIhfx lGiGGtVhfk lGiGGtVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH LgIlpL FJI FyVstL FyVstL FwVtpM dmssLnFyaI ftstLnFyaI	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSsi-F1 VhstASt-Fv VrttSSa-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv cAPlAPetwe sSPmAPetwe aGPiAPsget sGPiAPsget gSLFV11ft	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIh TgseYyIqIk SkleYyIqIl AqlpIvVqId AkmpVvVqIe AplhIsDtTk	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL lrqvVvdFdL vwrgPatFaQ vwrgPfsFeL ec-eapptIr kdasltle ngvtndla gtiptte ngtiptte ngtiptte ngtitle ngtitle ngtitle ngtitle ngttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdV QLfkyPGvyV QLfkyPGvyV QLfkyPGvyV QLfkyPGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s eqvprtia tlhsgdrs -ahlcre -ppmcrq dlslpsfe dlsvpsfp vilgled
GC GF Ar SL CL BL TR TC RF TE GC GF Ar SL CL BL TR TC RF TE GC GF Ar SL CL BL TR TC RF TC CL BL TR TC RF TC CL BL TR TC RF TC CL SL CL SL TR TC RF TC RF TC CL SL TR TC RF TC CL SL TR TC RF TC CL SL TR TC RF TC CL SL TR TC RF TC CL SL TR TC RF TC CL SL TC TC TC TC TC TC TC TC TC TC TC TC TC	MV LIV RV RV RV MV 213 RV MV 213 RV RV RV RV RV RV RV RV RV RV	rgFskPtLwV ttFesPrLhF ttFesPrLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV $\beta_{\rm g}$ ynf <u>Ggpvkh</u> - llf <u>G</u> lstvi- imp <u>G</u> tailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltl <u>G</u> qvmv- lll <u>G</u> qtvd- lvvPlnpllp vnl <u>G</u> faeprt h- <u>i</u> Ginfgsa $\beta_{\rm g}$ - <u>β</u> <u>G</u> - h-g <u>D</u> g <u>q</u> FeIa adg <u>Q</u> gFsLp k-sNeiFeVd t-lDakAtLq e-vNvdAsLq d-vDvvSsLk ee-D <u>g</u> SFeVk ee-D <u>g</u> SFeVk ee-D <u>g</u> SFeVa	<pre>IAAStAQLpw tCLTgNNKel tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yVLNtNDVac yUIDtNSLpa</pre>	sAQVtyrLea aADWqavVel aADWqavVel aADWqavVel aAEWgghIlw sDEWvytCev 	<pre>laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg gmkddanapy ciksalhats</pre>	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSi-Fl VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv gGIvGPkdsv cAPlAPetme aGPiAPsget sGPiAPsget gSLrFVllft SGVyAPkdcs	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd Image: hffDgarLd Image: hffD	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL vwrgPatFaQ vwrgPfsFeL /-0 ec-eapptlr kdasltle ngvtndla gtiptte gtvpetd gttmdwn ngvtnnwn andsysgwes ndnadgvefr dktahnle </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLagmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHv1L QAlrlPGcrI qvhppmfs-s egvprtia tlhsgdrs -ahlc-re -ppdc-re dlslpsfe dlsvpsfp yilgled dlppviatr-
GC GF Ar SL CL BL TR TC RR TE GC Ar SL CL BL TR TC RR TE GC Ar SL CL BL TR TC RR TE CC GF Ar SL CL BL TR TC RR TE CL BL TR TC RR TE CL BL TR TC RR TE CL BL TR TC RR TE CL BL TR TC RR TE CL BL TR TC RR TE CL SL TR TC RR TE CL SL TR TC RR TE CL SL CL SL TR TC RR TE CL SL TR TC RR TE CL SL TR TC RR TE CL SL TR TC RR TE CL SL TR TC RR TE CL SL TR TC CL SL TR TC RR TE CL SL TR TC CL SL TR TC CL SL TR TC CL SL TR TC CL SL TR TC CL SL TR TC CL SL TR TC CL SL TR TC TC CL SL TC TC TC TC TC TC TC TC TC TC TC TC TC	MV LW RV RV RV MV 213 RV MV 213 RV RV RV RV RV RV RV RV RV RV	rgFskPtLwV ttFesPrLhF ttFesPrLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV $\beta_{\rm g}$ ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- llGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa $\beta_{\rm g}$ k-sNeiFeVd t-lDakAtLq e-VNvdAsLq d-vDvvSsLk ee-DgsFeVk ee-DgsFalF sggDgeFsLp ptaGapFsTr	1AAStAQLpw tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yVLNtNDVac yVLNtNDVac gqivr -aakrqay -tsgrtay -ssgriay -gttkvy -gfdgaqwyp -vltgyapit rvvsgtntfy β G' IktRlhsaat IstP-haats LycPygenpt IqsPffatan IqsPfstan IqsPfyatan IrsPyhrtpa	sAQVtyrLea aADWqavVel aADWqavVel aADWqavVel aAEWgghIlw sDEWvytCev 	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGvGGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIfs lGvGGtIhfv lGiGGtNfk lGiGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt FH- EIJpL EIJpL EIfaT FYVstL FYIatL FYVstL FVVtpM dmssLnFyaT ftstLnFyaT avypLtDygF iptMaVclV	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>SGPiAPdahv</u> aGPfSPkdsv gGIvGPkdsv cAPlAPetme aGPiAPsget sGPiAPsget gSLrFVllft sGVvAPkdcs	<pre>isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLpkDgarLd -hffDgarLd -hffDgarLd -ffDgarLd -ffDgarLa -ggtVagw -fgtyVagw -fgtyVagar -ggtVeMgfggsyeFiVhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIh TgfqYyIhIh TgfqYyIAIA ApyrFmVyFd ApyrFmVyFd ApyrFmVyFd</pre>	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -Gfpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qvhppmfs-s eqvprtia tlhsgdrs ahlcre ppmcrq dlslpsfe dlsvpsfp yilgled qlppviatr-
GC GF Ar SL CL BL TR TC RR TE GC GF Ar SL CL BL Statutes	MV LWV RV RSV RV MV 213 RV MV 213 RV RV RV RV RV RV RV RV RV RV	rgFskPtLwV ttFesPtLhF ttFesPkLhF nsDhnGaFkV taFedVqFiL kgFadPvLlV $\beta_{\beta}C'$ ynfGgpvkh- llfGlstvi- impGtailh- vslAmrvds- vslAaklqs- lsfAvqqks- ltlGqvpmv- lllGqtlvd- lvvPlnpllp vnlGfaeprt h-iGinfgsa $\delta_{\beta} - \beta G - $ h-gDgqFeIa adgQgaFsLp k-sNeiFeVd t-lDakAtLq e-vNvdAsLq d-vDvvSsLk ee-DgsFeVk ee-DgsFaIe vdlRahRlLr sggDgeFsLp ptgGgpFsIr	lAAStAQLpw tCLTgNNKel tCLTgNNKel tCLTgNNKel vIIKdLENfe yVLNtNDVac yIIDtNSLpa $ -\beta C'$ = s-kkhai = sgnmstl = gqqivr = aakrqay = tsgrtay = sgrtay = gqtkvy = gfdgaqwyp = vltgyapit rvvsgtntfy $\beta G'$ IktRlhsaat IstP-haats LycPygenpt IqsPffatan IqsPffstan IqsPfyatan IrsPyhrtpa IrsPyhrtpa	sAQVtyrLea aADWqavVel aADWefvVel vADLgmeLsl aAEWgghIlw sDEWvytCev - αA SysravMSrn NanqalLRff TfprvvAAhf SyaagLShf SyaagLShf SyaagLShf SyaagLShf NlnstlVScv NfnntlLSyy ScsssiLEnh SfhqaaLSyf SfpaayTQll <u>teeGf</u> gr fledMl-r ftgqAngk fgdGta- fgdsG-ar rllaqSqs-r rlidaqSass ylmggsGslf rdrG fntG	laqgdeiahg yaeleeatsf yaefeaaksf fldvqdigmg qmkddanapy ciksalhats lGwSGtIsgs qGsNGtLhgr rSwTGkVrms lGvGGdIfs lGvGGtIhfv lGiGGtNfk lGmGGtVrgr lGiGGiVkgk rYwKGtLvle iSyGGtIhgr qSvGGiLhgt βH LgIlpL EJIpL FyIatL FyIatL FyVstL FyVstL FwVtpM dmsslnFyaI ftstlnFyaI avppLtDygF -iptMaVclv qLvIyaV	latrSiVtyP lg-kPtLvfD lg-kPnFiyS igpE elqlPvVP vankPiLslP VksvSSl-Fc IkkiGTa-Lt IqevSSi-Fl VhstASt-Fv VrttSSa-Fv VhicAPi-Fy VhvcSPc-Ty VifnLPa-Mg LvkiGSg-Lv VvqtGSk-Ai <u>sGPiAPdahv</u> aGPfSPkdsv gGIvGPkdsv cAPlAPetme cAPmAPetve sSPmAPetme aGPiAPsget sGViAPsget gSLrFV11ft sGVvAPkdcs gGPmGAqais	isleHlkdIe pgvfNgkfQf ldafNgslKf lpltD-sfLp kDgarLd -hffDgarLd l βE TasFvIfp-W TcsLlslrH TgtYMVgvsW ScSLRSValW TskLrIal-W TarLrVal-W SivLrVvseW GgtVeMgF QvdIaLam-W SceMfLilqP βI G <u>syeFi</u> VhIn AkyqFmCyFd GtfgFmIhIh TgfqYyIhIh TgfqYyIAIh TgfqYyIAIh TgfqYyIAIh AglpIvVqId AkmpVvVqIe AplyFmVyFd ApyrFmVyFd ApyqYmVhFs	<pre>imlpPrqMaI ltcpPifFdL ltipPleYdL urqvVvdFdL vwrgPatFaQ vwrgPfsFeL </pre>	-Gnagsinfp tAvtalrsag sAtsayksvs sTtt-pkgka -G-fpstin -Grss-kren CB βF QVlwgPHqiM TAyqrPHyiL DIvtrKHwiV ELaqiPHvdV QLaqmPHvdV QLaqmPHvdV QLsqmPHvdC ELfkyPGvyV QLfkyPGcyI DAyryFGstV ELikiPHvlL QAlrlPGcrI qVhppmfs-s eqvprtia tlhsgdrs -ahlc-re -ppmc-rq dlslpsfe dlsvpsfp yilgled qlppviatr- dppprpi

Figure 6 continued

	352	? B-A doma	in-linking r	polvpeptide	I8BI		I-BC	r– I	BC "
	TRSV	sEl			VnWftltnlk	nDan	ta-wynfd	TPGVThDFag	kdatytLa
	TORSV	aE			EnWosfrnfk	b <u>ban</u>	wkfo	WPARLopTid	dkeevillr
	PPCV	y <u>n</u>		44	SaWfrinni-	aDdn	wkre	TPGPTeDTia	
		iNr		ngh	FaWfmlohl	Dtn	tt a pralk	IPGRIEDIIa IDARMONIta	k orgit
	IBRV-ED	iNr.		lign	Fawrmieni-	Dun	also nikuk	IPARMANLUS	kevqvt
	IDRV-5	iNy		kqi	Fawcileci-	D-11	kas-pikvk	IPSKIGNLSS	
	GCMV	dDv		dđr	Fawitiiip=	ppp-	-KISKIIKIU	LPSRVCNIAy	ReatVt
	GFLV	aDy			Fvwvdiseit	IDke	ere	IGSRFIDFUS	ntervsil-
	ALMV	nDy			FIWVAISSIL	livlylob	eyv	IGSRFIDISS	lLSLVal-
	SLRSV	CEyiqpttsi	grinpativa	sgapvvqvgt	Sawmepplir	lipigiiQk-	-ĸIrii	TISKWpKSgi	lipmtPssn
	CLRV	1Q			Fiwasisgi-	apvvpEa-	-rtwm	IPCRLSDYKV	egatLkm-
	BUWA	gNv1		T	Fnwatisemt	NIC	rīd	IPARLSDLV1	pgqtvtm-
	394	l (XA-	βD		βe-	-β	β F- βG	I.
	TRSV	<u>s</u> NPls <u>w</u>	LV <u>A</u> a-t <u>G</u> why	GeVdLcisWs	r <u>skq</u> a- <u>qa</u> q-	<u>e</u> GsVsittny	- <u>rdwgayw</u> q <u>G</u>	q <u>a</u> r <u>iy</u> dlrrt	eaei
	ToRSV	qHPlsl	LISs-tGfft	GrAiFvfqWg	lnttagnmk-	-GsFsaklaf	gkgveeieqT	stvqplvgac	eari
	RRSV	nyvNPtsl	LFSvGlhg	GiIrLhitWc	pnttlgesk-	-GtLkymqyl	yhtatenffG	dqatrgiiid	qdgfti
	TBRV-ED	nfvNAlai	MCAt-tGmhf	<u>GkCtLhfsWg</u>	wyrgkladqs	-GaVslqtgm	graavaehfG	gkhnfi-cyp	atsfsl
	TBRV-S	nfv <mark>NA</mark> lai	LCAt-tGmhh	GnCtIhfsWl	whpaelgkql	-GrLkfvqgm	ginnehiG	dtmcynsl	snthsv
	GCMV	ge <mark>NP</mark> faa	MIAc-hGlhs	GvLdLklqWt	lnkgtsfkdl	qGhIsfysgm	gdstigehhG	efhlggpl	ssslav
	GFLV	nyv <mark>NA</mark> fai	MCAt-tGmha	GkCiLhfsWs	ln-tefgkss	-GsVtitklv	gdkamgldgP	shvfaiqkle	gtt
	ArMV	gdNPfah	MIAc-hGlhh	GiLdLklmWd	le-gefgkss	-GgVtitklc	gdkatgmdgA	srvcslqnmg	cet
	SLRSV	mpklvGTfeg	EVEq-hSplm	HrSqEnaqWc	gsltyylsir	ySgAtpqgvl	pmrpvcfgaT	vldnildkpc	fvekdtfiqv
	CLRV	eaHPlar	LVAsirGmfq	GtMrFilrWt	fasslttpt-	tyvqlv	hkfgtT	tsses	yltk
	BLMV	rr-NAlan	LIRsc-Gffr	GrVtFvfqWt	lnvahivpta	tMqIltavgr	vgnaetngsQ	ilqwivpvsq	vfekev
	460)		βн	-	βI	βı		
	TRSV	piflGs	<u>YAG</u> a <u>T</u> ps	galgkqnyVr	isivnakdiv	aLrVcLrpk-	SIkFWGRSat	lf	
	ToRSV	pvefKt	YTGyttsGpp	gsmepyiyVr	-tt-qaklvd	rLsVnVilqe	GFsFYGPSvk	hfkkevgtps	atlgtnnppv
	RRSV	diacGd	FFGatrvGlp	geverlgiY-	iss-naksia	eIrVsFev-1	SMnFYGSTik	vtav-ggfpi	lkpptpapsi
	TBRV-ED	pfqfGs	FAG pisc G ga	pma-aenw⊺e	liipnmkwit	sLtVsIevhd	GFqFYGRSag	pltipa	
	TBRV-S	pfqf <mark>G</mark> s	FAGpitsGgk	ade-aenw ⊺ e	iqspdfswva	sLhVsIevhe	GFkFYGRSag	pltipatvad	vsavsgs
	GCMV	pfef <mark>G</mark> s	FAG pvts G gt	pft-senwLr	vetahwdwlt	sLtVdIqvlp	GFrFYGRSag	pltip	
	GFLV	ellvGn	FAGanpnTrf	slysrwmalk	ldqaksik	vLrVlCkprp	GFsFYGRTsf	plv	
	ArMV	elyiGn	YAG anpnTal	slysrwlaIk	ldkaksmk	mLrIlCkprg	NFeFYGRTcf	kv	
	SLRSV	mpmadpreTi	YL-ptteGva	fyetprrw∨n	thfgates-y	gVrTcPawv-	LLqFPNEEas	hlgvrdvslw	vepnisfrh-
	CLRV	Lr	HAS	qstvLn		-LdVvVaglg	GF-MRLRSg-		
	BLMV	emdlTd	YPGfnts <mark>G</mark> gi	gadhdqpyId	iacgnfpqif	yMnInVrvhp	GFeLYGRSit	plri	
	mp (177								
	TRSV			1					
	TRSV ToRSV	grpp-envdt	ggpggqyaaa	lqaaqqagkn	pfgrg				
	TRSV ToRSV RRSV	grpp-envdt dyyyientfp	ggpggqyaaa vg	lqaaqqagkn	pfgrg				
	TRSV ToRSV RRSV TBRV-ED	grpp-envdt dyyyientfp	ggpgggyaaa vg	lqaaqqagkn 	pfgrg 				
	TRSV ToRSV RRSV TBRV-ED TBRV-S	grpp-envdt dyyyientfp	ggpggqyaaa vg	lqaaqqagkn 	pfgrg 				
	TRSV ToRSV RRSV TBRV-ED TBRV-S GCMV GELV	grpp-envdt dyyyientfp	ggpggqyaaa vg	lqaaqqagkn	pfgrg 				
	TRSV ToRSV RRSV TBRV-ED TBRV-S GCMV GFLV ArMV	grpp-envdt dyyyientfp	 dabaadAssa	lqaaqqagkn	pfgrg 				
	TRSV ToRSV RRSV TBRV-ED TBRV-S GCMV GFLV ArMV SLRSV	grpp-envdt dyyyientfp	 dabaddAssa	lqaaqqagkn	pfgrg 				
	TRSV TORSV RRSV TBRV-ED TBRV-S GCMV GFLV ArMV SLRSV CLRV	grpp-envdt dyyyientfp	Additional contraction of the second	lqaaqqagkn	pfgrg 				
	TRSV TORSV RRSV TBRV-ED TBRV-S GCMV GFLV ArMV SLRSV CLRV BLMV	grpp-envdt dyyyientfp	Additional and a second	lqaaqqagkn	pfgrg 				
Stru	TRSV TORSV RRSV TBRV-ED TBRV-S GCMV GFLV ArMV SLRSV CLRV BLMV	grpp-envdt dyyyientfp		lqaaqqagkn	pfgrg 				

Amino acid sequence alignment of eleven nepovirus capsid proteins. The secondary structure features and residue numbers for TRSV, based on the structure reported here, are indicated above its sequence. Residues that are conserved among at least seven nepoviruses in the C, B and A domains are shown in green, red and

Most of the loops connecting the β strands in nepoviruses show some variations with at least one or two amino acid insertions or deletions. This is in contrast to comoviruses, where the insertions and deletions are blue upper case letters, respectively. Underlined residues in the TRSV sequence occur on the outer surface of the capsid. Upper case black letters correspond to residues that are not conserved in columns where seven or more residues are conserved.

confined to the BC and DE loops on the exterior surface of the capsid near the fivefold axes. The regions with the greatest variation in the three domains of TRSV include the loops present on the external surface of the capsid, the two interdomain linking polypeptides and the N and C termini. A subset of nepoviruses comprising of strawberry latent ring spot virus (SLRSV), CLRV and blueberry leaf mottle virus (BLMV) vary the most relative to the other nepoviruses.

Most of the conserved hydrophobic residues are present in the strands forming the β -barrel with their sidechains contributing to the hydrophobic core in the three domains. Several externally exposed loops contain conserved residues interspersed with insertions and deletions.

Some of the sequence conservation may stabilize the quaternary structure of the three-domain subunit. The sequence motif Phe-Tyr-Gly-Arg-X-Ser (FYGRXS) present in the β I strand in the A domain interacts with a conserved stretch of residues (Pro177 to Pro182) in the C-B domain-connecting polypeptide. SLRSV is the only nepovirus that lacks both of these conserved regions. This suggests that the motif in the A domain in nepoviruses is important for maintaining the rigid structure of the C-B domain connector. Constraining the flexibility of this extended polypeptide may be important for the assembly of the nepovirus capsid proteins. As predicted previously [25], the sequence motifs FYGRXS in the A domain and Phe-Asp-Ala-Tyr-X-Arg (FDAYXR) present in the βE strand in the C domain occur adjacent to each other in the structure of TRSV. The motif present in the A domain is involved in stabilizing the quaternary structure of the domains within the capsid protein, while the motif in the C domain is accessible from outside the capsid and this region on the viral surface may have some functional significance in the nepoviruses.

SLRSV is characterized as a nepovirus based on nematode transmission specificity. Unlike the other known nepoviruses, the processing of the capsid polyprotein of SLRSV is the same as for comoviruses (i.e. it is cleaved into two capsid protein subunits by a virally encoded protease at a specific site in the B-A domain-connecting polypeptide). When the length of the N terminus and the two domain-connecting polypeptides in TRSV are compared to other nepoviruses, only SLRSV has a longer N terminus, a shorter C-B domain connector and a much longer B-A domain connector. It is interesting that in these three trends SLRSV closely resembles comoviruses. The sequence Ser-Gly, recognized by the SLRSV protease, occurs at five different locations along the SLRSV capsid polyprotein. Three of these sites will occur on exposed loops based on the TRSV structure, but the protease cleaves only at the site in the B-A domain-linking polypeptide. This observation indicates that the presence of the cleavage site in an extended chain must be essential for cleavage by the SLRSV and comovirus proteases. A similar trend has been observed

in picornaviruses based on their three-dimensional structures [26].

Surface amino acid conservation in nepoviruses

The variability of all the surface residues in the structure of TRSV was examined using the program GRASP [27] (Figure 7). A few residues are conserved among at least nine nepoviruses; these are confined to two regions, site 1 and site 2, on the TRSV surface. Site 1 is located in a minor depression at the junction of the A and C domains between the pseudo-twofold and the pseudo-threefold axes (Figures 5a and 7). Site 1 is comprised of residues from several regions: the EF loop in the C domain (including Asp79 present in the FDAYXR sequence motif); the β C- β D insertion in the A domain; and the GH loop in all the three domains. Site 2 occurs on a minor surface protrusion, close to the icosahedral threefold axes, where the conserved residues on the BC and HI loops in the B and C domains form a ring.

The nematode species that are required to transmit ten of the 11 nepoviruses shown in the sequence alignment in Figure 6 are known. The nepoviruses, with the exception of BLMV, fall into four groups based on the nematode species specificity: TRSV and tomato ringspot virus (ToRSV); raspberry ringspot virus (RRSV) and tomato blackring virus (TBRV); grapevine chrome mosaic virus (GCMV) and grapevine fourleaf virus (GFLV); and arabis mosaic virus (ArMV), SLRSV and CLRV. Regions





Depth-cued view of the external molecular surface of a pentamer of TRSV subunits. The figure shows the variability of the surface residues among nepoviruses. The surface residues in TRSV are color-coded according to their conservation number, which denotes the number of nepoviruses in Figure 6 containing a similar residue at that position. The color-code ranges from red (conservation number 1) through yellow (conservation number 6) to green (conservation number 11). Two regions, site 1 and site 2, containing some residues conserved among at least eight nepoviruses are indicated. (The figure was generated using GRASP [27].)

Stereo view C α traces of the capsid proteins of TRSV superpositioned on the capsid proteins of BPMV. The superposition is viewed from outside the capsid. The C, B and A domains in TRSV are shown in green, red and cyan, respectively; the C and B domains of BPMV are shown in orange and the A domain is in purple.



on the viral surface that correlate with the nematode transmission specificity of the four groups of nepoviruses could not be identified on the basis of the surface amino acid variability. Nepoviruses transmitted by the same nematode species do not share regions of similar amino acid composition on the viral surface. This also suggests that the conserved site 1, containing Asp79, on the viral surface is not directly involved in determining vector specificity. This site may serve as a binding site for some unknown host factor or viral protein. Further mutation studies are required to identify the functional significance of site 1 in the nepovirus life cycle. The significance of the ring of conserved residues around the threefold axes (site 2) is also not clear. This region in CPMV constitutes a major antigenic site, as demonstrated by the cryo-EM structure of a complex formed between an Fab fragment of a monoclonal antibody raised to CPMV and the virus [28,29].

Structural similarity to comoviruses

Structural superposition of the capsid proteins of TRSV and the comovirus BPMV was performed using the least squares superposition option in the graphics package O [30]. Based on the hydrogen-bonding pattern of the eight β strands forming the β barrel, the corresponding stretches of residues in the β strands of TRSV and BPMV were identified for superposition. The regions with the greatest deviation in distance between equivalenced C α positions in TRSV and BPMV include the external surface loops, the two domain-connecting polypeptides and the N and C termini (Figure 8).

The sequence alignment of the C, B and A domains of TRSV and three comoviruses (BPMV, CPMV and RCMV), based on structure superposition, is shown in Figure 9. The three comoviruses are structurally very similar (W Wikoff, T Lin and JEJ, unpublished data).

Most of the β strands forming the core β barrel in BPMV are longer than the corresponding strands in TRSV. About 58 of the 513 residues in TRSV are conserved among at least seven nepoviruses and the comoviruses. Most of these residues are hydrophobic and occur on the β strands and α helices with their sidechain buried in the hydrophobic core. Among the non-glycine and non-proline residues that are conserved, it is intriguing that the residues Asp79 and Arg83, present in the FDAYXR sequence motif in the C domain of TRSV, are also conserved as Asn3093 and Arg3097 in comoviruses. This indicates that the function of the conserved site 1 in nepoviruses may also be shared by comoviruses.

The statistics for the structural superpositions (Table 1) support the proposed evolutionary connections between the capsid polyproteins of the three groups in the picornavirus superfamily. Among the three equivalent domains in TRSV and BPMV, the B domains display the most structural similarity while the A domains exhibit the least (Table 1). The superposition of the three different domains within TRSV or BPMV onto each other indicates that the B and C domains are structurally the closest in both the viruses (Table 1). A similar trend has been observed among picornaviruses [21,31]. The equivalent domains in TRSV and BPMV are structurally closer than the three different domains within TRSV. Furthermore, the structures of the three β -barrel domains in comoviruses have diverged from each other slightly more than the domains in TRSV. The three β -barrel domains within a picornavirus [21], on the other hand, have clearly diverged much more than those in either comoviruses or TRSV. The comparisons suggest that the three domains in the nepovirus capsid protein diverged significantly before the comovirus polyprotein, with its cleavage site in the B-A domain-linking polypeptide, evolved.

Amino acid sequence alignment of the capsid proteins of TRSV and three comoviruses (CPMV, RCMV and BPMV) based on the structural superposition. The secondary structure elements and sequence numbers of TRSV are given on the top line. Residues conserved among at least seven nepoviruses (shown in Figure 6) and the three comoviruses in the C, B and A domains are shown in green, red and blue bold upper case letters, respectively. Residues that are conserved among TRSV and any of the three comoviruses in the C, B and A domains are shown in upper case and in green, red and blue, respectively. Residues in lower case letters were not observed in the structures of comoviruses.

-BB---| $|--\beta C-|$ -αA'--αA" --CGTLSFKVPK DAKKGKHLGT FDIRQAIMDY TRSV AVTVVPDPTC GGLHSOEWCA MEQNLFALSL DDTSSVRGSL -----LDTK FAQTRVLLSK AMAGGDVLLD EYLYDVVNGQ DFRATVAFLR TDTDLFKLSL DDTSSPKGSL -----LDTR FAQKKVLIPK AMAGGADLLS SNLYDVLSGS SFRASLALAR CPMV RCMV BPMV METNLFKLSL DDVETPKGSM -----LDLK ISQSKIALPK NTVGGTILRS DLLANFLTEG NFRASVDLQR 3001 ---βB---| |--βC--||-αA'| αA" |-αB| 50 |--BD--| $|--\beta E-|$ $|\beta_{\rm F}|$ |BG-| TRSV KGIVNPTFTV RMHAPRNAFA GLSIACTFDD YKRIDLPALG NECPPSEMFE LPTKVFMLKD ADVHEWQFNY CPMV THVITGKIKV TATTNISDNS GCCLMLAINS GVR--GKYST -DVYTICSOD SMTWNPGCKK N--FSFTFNP THVVEGKIRC ICTINLPENT GCCLAITVNS SNR--GQFST -DIYTTGSQD RILWNPACSK N-RCMV -CDFSFNP CELWNPACTK A--MTMSFNP BPMV THRIKGMIKM VATVGIPENT GIALACAMNS SIR--GRASS -DIYTICSQD 3064 |-βD--|--BE--| |-BF-| | BG- | |-αB-| |-BH--| |--BI--| 121 βG C-B linking polypeptide GELTGHGLCN WANVATQPTL YFFVASTNQV TMAADWQCIV TMHVDMGPVI ----DRFELN PTMTWFIQIG NP-CGDSWSA EMISRSRVRM TVICVSGWTL SPTTDVIAKL DWSIVN---- EKCE---PT -IYHL-ADCO TRSV CPMV RCMV NP-CGTAWSL EFLRRTKFHL SVTCVSGWSA QPQTDIAMTM DWYVSN----KPCP----VC -IYNV-GTPG BPMV NP-CSDAWSL EFLKRTGFHC DIICVTGWTA TPMQDVQVTI DWFISS----OECV----PR -TYCV-LNPO 3130 -βн--BI---| C-B linking polypeptide |--βC' |----βB-----|-BC| |βC"||-αA--| βD' | -- βD--186 TRGV GDTFAIDRYY EAKEIK-L-D GSTSMLSISY NFGGPVKHSK KHAISYSRAV MSRNLGWSGT ISGSVKSVSS QNWLPLNRWM GKLTFPQGVT SE-VRRMPLS IGGG-AGATQ AFLANMPNSW ISMWRYFRGE LHFEVTKMSS CPMV GONVWVNRWM GKLSFPQGSQ NQ-LKQMPLA IGGG-AGAKN SILMINMINAF LSLWRYFHGD LVFEVQKMSS ONPFVLNRWM GKLTFPQGTS RS-VKRMPLS IGGG-AGAKS AILMINMPNAV LSMWRYFVGD LVFEVSKMTS RCMV BPMV 2006 --βB |βC| βC ' βc" -αA-·βD |---βE-- $|--\alpha B-|$ $|-\beta F|$ -βg-| 254 βG LFCTASFVIF PWECEAPPTL RQVLWGPHQI MHG----DG QFEIAI--K- TRLHSAATTE E-----G PYIKATVTFL IAFGNLSDAF GFYESFPHRI VQFAEVEEKC TLVFSQQE-F V--TAWSTQV NPRTTLEADG TRSV CPMV PFIKSTVTFF IGFGGLPF-S ENLEDFPNKL IQFGEVQERV EITFTRKE-F L--TAWSTQV DPAGPVAGDG PYIKCTVSFF IAFGNLADDT INFEAFPHKL VQFGEIQEKV VLKFSQEE-F L--TAWSTQV RPATTLLADG RCMV BPMV $|\alpha B - | | -\beta F|$ 2074 |--βe--|--βG--| 308 |----βн--------βI----| B-A domain-linking 349 TRSV CPMV RCMV BPMV CPYLYAMVHD SSVST--IPG DFVIGVKLTI IENM-C-AYG LNPGISGSRL LG--B-A domain-linking 2141 |--BH-| |----|| 2192 eptide |----βB---| |-βC| -- --SSSELYNW FTL-TNL--- -K-P-DA--N TGVVNFDIPG YIHDFASKD-350 polypeptide BC" -ATVTLAS TRSV GPVCAEAS-- ---- DVYSP CMIASTPP-A P-F-S---D --VTAVTFDL -INGKITPVG DDNNNTHIYN GGVVRTTD-- ----GVYST CFRVRTPL-A L--K---D --SGSFTCDL -IGGGITTDS NTGWNLTALN CPMV RCMV GGVVRTTD-----SI SQQ--TVWNQ MATVRTPLN---F--DSSKQ S-FCQFSVDL BPMV -LGGGISVDK TGDWITLVQN 1001 polypeptide |--βB--| |--BC-| BC ' BC' 395 |--0A--|----BD--|-βE- $|-\beta F - |$ $1 - \beta G$ WQGQA RIYD--LRRT TRSV NPLSWLVAAT GWHYGEVDLC ISWSRS--KQ AQAQEC SVSI TTNYRD-WGAY PPINNVLRTA AWKSGTIHVQ LNVRG-AGVK RADWDGQVFV YLRQSNNPES TPVANLLRTA AWKRGTIHVQ VAMFG-STVK RSDWTSTVQL FLRQSMNTSS SPISNLLRVA AWKKGCLMVK VVMSGNAAVK RSDWASLVQV FLTNSNSTEH CPMV ---YDARTFV ISO-PGSAML ---YDARVWV ISK-PGAAIL RCMV BPMV ---FDACRWT KSE-PHSWEL 1054 |---αA--| |----| |--BE--| |--βF-| $-\beta G$ 456 |βH| -BI----| |BI'| 513 TRSV EAEIPIFL-- ----GSYAGA TP-S---GA- LGKQNYVRIS IVNAKDIVAL RVCLRPK-SI KFWGRSATLE NFSFDIIGPN SGFEFA---E SPW-ANQ-TT W-YLECVA-T NP-RQIQQ-F EVNMRFDPNF RVAGNILMPP EFSFDVEGPN NGFEMW---E ANW-ASQ-TS W-FLEFLI-S NV-TQNTL-F EVSMKLDSNF CVAGTTLMPP CPMV RCMV BPMV IFPIEVCGPN NGFEMW---S SEW-ANQ-TS W-HLSFLV-D NP-KQSTT-F DVLLGISQNF EIAGNTLMPA 1120 -BG--| |----βI----|----BH---TRSV CPMV FPLSTETPPL lkfrfrdier skrsvmvght ataa RCMV FSVTaspdsr pllgvktstp akkyvggslg agpspd BPMV FSVPOanars senaessa-1181 1198 Structure

Electron density in the RNA region

The structure of BPMV revealed the interaction of a trefoil-shaped cluster of RNA with the cleft between the covalently linked B and C domains in the capsid protein near the icosahedral threefold axes. Based on the observed protein–RNA interactions, it was proposed that the RNA in comoviruses may be recognizing a preformed icosahedral binding site in the assembling capsid [7]. Except for a piece of density that has been modeled as a nucleotide near the VP2 subunit, ordered RNA density has not been observed in the mature picornavirus structures [14,15,31]. The crystal structure

of the P1 poliovirus empty capsid, however, revealed a trefoil-shaped depression on the inner surface of the capsid that was strikingly similar to the RNA-binding site in BPMV [32].

The inner surface architecture of the cleft between the B and C domains in TRSV is quite different from the RNAbinding pocket in BPMV due to differences in the conformations of the C–B domain-connecting polypeptide and the N terminus (Figure 8). There is no evidence for the presence of density corresponding to well-ordered RNA in this cleft in TRSV.

Structural	Superimposed pairs		Root mean	Number of equivalenced	Percentage equivalence (%) ⁺⁺		
comparison*	Domain 1	Domain 2	square deviation	residues ⁺	Domain 1	Domain 2	
TRSV versus	TRSV C	BPMV C	2.0	123 (129)	84 (72)	80 (71)	
BPMV	TRSV B	BPMV B	1.4	127 (137)	83 (81)	78 (71)	
	TRSV A	BPMV A	2.2	119 (122)	75 (74)	70 (66)	
TRSV versus	TRSV A	TRSV B	2.3	81	58	57	
TRSV	TRSV B	TRSV C	1.8	78	56	52	
	TRSV C	TRSV A	2.2	78	50	55	
BPMV versus	BPMV A	BPMV B	2.3	73	46	46	
BPMV	BPMV B	BPMV C	2.0	82	54	57	
	BPMV C	BPMV A	2.2	79	54	51	

Root mean square deviation (σ) in C α displacements (Å) for residues in the core β strands and loops connecting the strands in the pairs of superimposed domains.

*TRSV = tobacco ring spot virus; BPMV = beanpod mottle virus. [†]The numbers given in parentheses refer to the superposition of all the residues (including the N and C termini) in the pair of domains. [‡]The percentage equivalence shown in the last column is given by [number of equivalenced residues/total number of residues in the β -barrel domain] × 100.

Biological implications

Tobacco ringspot virus (TRSV) is a member of the nepovirus genus of icosahedral RNA plant viruses. Nepoviruses cause diseases in fruit crops, have a wide host range and are transmitted by adult and larval stages of soil-inhabiting eel worms (nematodes). Plant virus families are classified into superfamilies based on genetic similarities to animal viruses. Nepoviruses, comoviruses and picornaviruses constitute members of the picornavirus superfamily that have icosahedral capsids. The capsids of the icosahedral picorna-like viruses are composed of 60 copies of three capsid protein subunits (each with a similar β -barrel fold), which occupy positions in the surface lattice comparable to those of 180 copies of a single capsid protein subunit in a T = 3 virus capsid. The three different subunits are synthesized as a polyprotein, which is subsequently cleaved by a viral proteinase.

Previous structural studies of members of the picornavirus superfamily have suggested that picornaviruses evolved from a T = 3 virus by triplication of the gene encoding a β -barrel domain, followed by the independent evolution of the three β -barrel domains and the development of cleavage sites in the interdomain-linking regions in the capsid polyprotein. The picornavirus capsid polyprotein is cleaved at two sites to yield three subunits. The comovirus polyprotein, on the other hand, is cleaved at only one site to yield a large subunit with two β -barrel domains and a small subunit with one β -barrel domain. The capsids of nepoviruses are composed of 60 copies of a single capsid protein composed of three β -barrel domains and lack any cleavage sites. As such, the nepoviruses may represent an early stage in the evolution of picornavirus capsids. Structural studies on TRSV were initiated to investigate the proposed evolutionary connection among the three groups in the picornavirus superfamily.

The crystal structure of TRSV has revealed that the capsid protein subunit is folded into three β-barrel domains that are covalently linked together by extended polypeptides as 'beads on a string'. The observed order of connectivity of the three domains from the N to the C terminus in the capsid protein subunit is consistent with the proposed connectivity for the precleaved comovirus and picornavirus capsid polyproteins. The three different domains within TRSV and comoviruses are more closely related at the structural level than the three domains within picornaviruses. The results of structural comparison and a sequence alignment among nepoviruses and comoviruses support the notion that the capsid polyproteins of nepoviruses, comoviruses and picornavirus have evolved from a common ancestor via divergent evolution. The first structure of a nepovirus also provides a snapshot of how the development of cleavage sites in the capsid polyprotein yields new flexible N and C termini which can intertwine and stabilize the quaternary interactions between the subunits during capsid assembly.

Nepoviruses exhibit diversity in the capsid protein sequence and nematode species specificity during transmission. Previous studies have indicated that retention of the nepovirus capsid in the gut lining of the nematode vector dictates virus vector specificity. Nepoviruses transmitted by the same nematode species do not share regions on the viral surface with similar amino acid composition. A few residues on the TRSV capsid surface, clustered in two regions, are conserved among nepoviruses. Further studies are needed to understand the role of these regions as potential binding sites for host factors or viral proteins involved in either cell-to-cell movement or some other function shared by all nepoviruses.

Materials and methods

Crystals, data collection and processing

TRSV was propagated in tobacco plants, purified by a modified procedure reported by [33] and crystallized at room temperature using 2-3% polyethylene glycol (PEG) MW 3350 as precipitant. The virus sample used for crystallization contains empty capsids, particles with one molecule of RNA1 or RNA2 and particles containing two molecules of RNA2. X-ray diffraction data were collected at the Cornell High Energy Synchrotron Source (CHESS) on the F1 beam line ($\lambda = 0.91$ Å, F = 300mm) employing Fuji image plates. The TRSV particles crystallize in the monoclinic C2 space group (a=407.1 Å, b=399.7 Å, c = 285.9 Å and $\beta = 129.1^{\circ}$) with half a virion in the crystallographic asymmetric unit giving 30-fold noncrystallographic redundancy. The crystals diffract X-rays to 3.3 Å and the diffraction data were only 22% complete to 3.5 Å resolution with an $R_{\rm merge}$ of 10.3%. The orientation of the virus particle in the unit cell was determined by rotation function analysis [34]. It was not possible to generate a more complete data set at this time because of problems associated with obtaining crystals of TRSV that diffract X-rays to high resolution consistently.

Structure determination and refinement

The structure of TRSV has been determined to 3.5 Å resolution using only the 22% complete data. A data set composed of 22% of the crystallographically unique reflections corresponds to nearly sevenfold noncrystallographic redundancy (100% of the data corresponds to 30 icosahedral asymmetric units). The power of real-space electrondensity averaging allows this redundancy to be exploited explaining the success in solving the structure [35]. The polyalanine model for the three β -barrel domains in CPMV [7] were treated as three independent rigid bodies and subjected to XPLOR rigid-body minimization [36] using all the 4045 observed unique reflections for TRSV in the resolution range of 20 to 10 Å. After 40 cycles, the XPLOR R factor dropped from 56.5% to 49.5%. The XPLOR R factor = $[\Sigma|F_o - F_c|/\Sigma F_o] \times 100$, where $\rm F_{o}$ and $\rm F_{c}$ are the experimentally observed and atomic model based calculated structure-factor amplitudes, respectively. The phases calculated from this model were combined with the observed structurefactor amplitudes for TRSV in the 20 to 10 Å resolution range to generate an electron-density map.

Real-space electron-density map averaging and solvent flattening were performed using the programs in the RAVE and CCP4 suites [37]. An atomic mask, based on the coordinates of the initial phasing model using MAMA [38], was used for averaging. After 15 cycles of averag- $R_{MR} = [\Sigma|F_o - F_c|/\Sigma F_o] \times 100$, where F_o is as defined above and F_c is the structure-factor amplitude obtained by inverse Fourier transformation of the modified electron-density map. Phase extension from 10 Å to 3.5 Å resolution was carried out in 42 extension steps with a step size of one reciprocal lattice point and involved 630 cycles of phase refinement by averaging. Using the package O [30] a structural model containing all 513 residues present in the capsid protein of TRSV [5] was built to the 3.5 Å resolution electron-density map. Of the residues present in the solvent accessible surface loops on the capsid, 23 of these, including Lys17, Lys23, Arg83, Asn91, Glu92, Glu100, Lys109, Asp110, Asp112, Asn130, Arg172, Glu174, Lys201, Leu202, Asp203, Arg274, Glu275, Glu330, Arg339, Lys386, Lys421, Trp443 and Arg482 have ill-determined sidechain density due to higher sidechain mobility. A new atomic mask based on the 3.5 Å structural model of TRSV and an inner radial cut-off of 70 Å was generated. Ten cycles of averaging at 3.5 Å were carried out using the new mask and phases from the structural model, in order to locate any regions of ordered RNA that may be interacting with the protein shell.

The structural model was subjected to conjugate gradient energy minimization using XPLOR [36], followed by manual rebuilding. After 300 cycles of positional refinement using all the reflections between 8 Å to 3.5 Å, the R factor decreased from 31.4% to 26.9%. Further refinement of the structure has not been carried out due to the incompleteness of the diffraction data. The present structure of TRSV has an R factor of 28.5% for all the measured reflections between 20 Å and 3.5 Å resolution. The root mean square (rms) deviations in bond distances and bond angles are 0.011 Å and 2° , respectively.

Buried surface area calculation

The buried surface areas reported in Figures 5b–5d are based on the accessible contact surface areas calculated for the various domains and domain interfaces in the capsid, using a probe radius of 1.4 Å in the program ACCESS [39]. For instance, the difference between the accessible surface area for one intact subunit (containing three domains) and the sum of the accessible surface areas for the A, B₅ and C domains as separate modules will yield the buried surface area for the single subunit.

Accession numbers

The coordinates for the TRSV capsid protein will be deposited in the Brookhaven Protein Data Bank.

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