cis-acting hydrolase element ("Chysel")

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MI/BCH/BIO 615

Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'.

Donnelly ML, Luke G, Mehrotra A, Li X, Hughes LE, Gani D, Ryan MD.

J Gen Virol. 2001 May;82(Pt 5):1013-25.

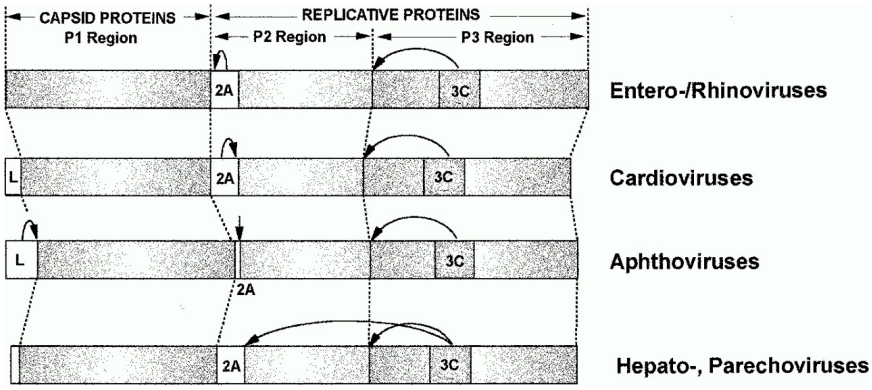


Fig. 1. Picornavirus 'primary' polyprotein cleavages. The genomes of picornaviruses are shown with the single, long, ORF in boxed areas. Primary, intramolecular cleavages (in cis) are shown (curved arrows).

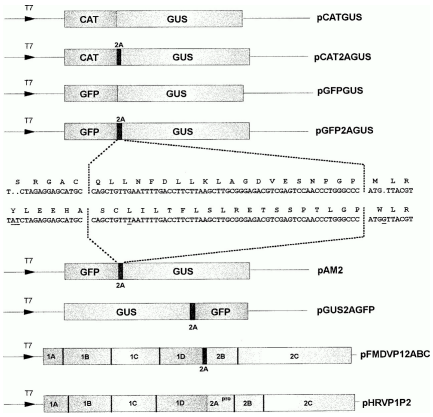


Fig. 2. Plasmid constructs. Regions of plasmids encoding polyproteins (boxed areas) are shown. The sequence of the 2A region of pGFP2AGUS is shown for comparison with that of pAM2. Frame-shift and point mutations are underlined.

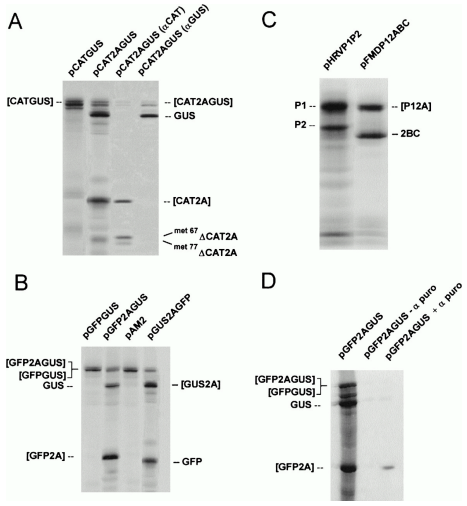


Fig. 3. Translation in vitro. Translation products from pCATGUS and pCAT2AGUS are shown together with immunoprecipitated products (A). The translation products from pAM2 are compared with those of pGFPGUS, pGFP2AGUS and pGUS2AGFP (B). (C) Translation products derived from the control constructs pHRVP1P2 and pFMDP12ABC. (D) pGFP2AGUS was translated in the presence of puromycin (50 µg/ml) and the translation products were then immunoprecipitated using null serum (-puro) or anti-puromycin antibodies (+puro).

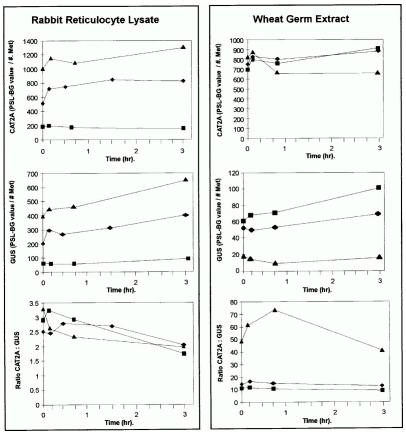


Fig. 4. Rates of protein degradation in rabbit translation systems in vitro. Data from three independent experiments are shown (, expt 1; , expt 2; , expt 3). for each translation system. Phosphorimaging data (photo-stimulated luminescence; PSL) were corrected for methionine contents, the ratio PSL [CAT2A]:PSL [GUS] being shown for each experiment.

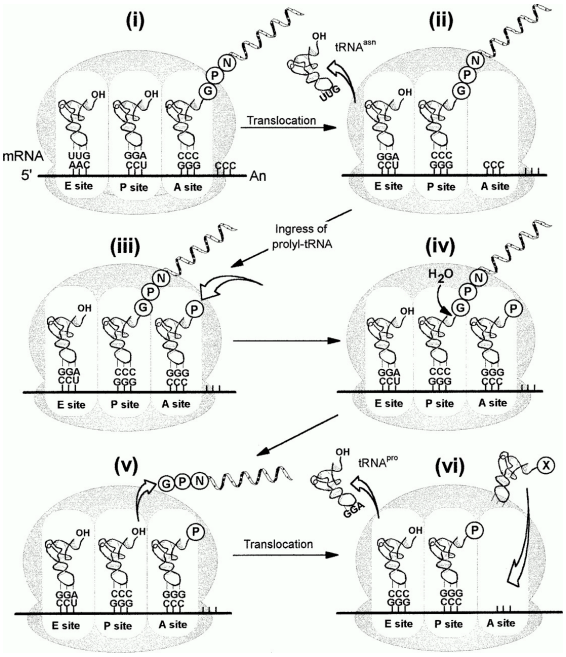
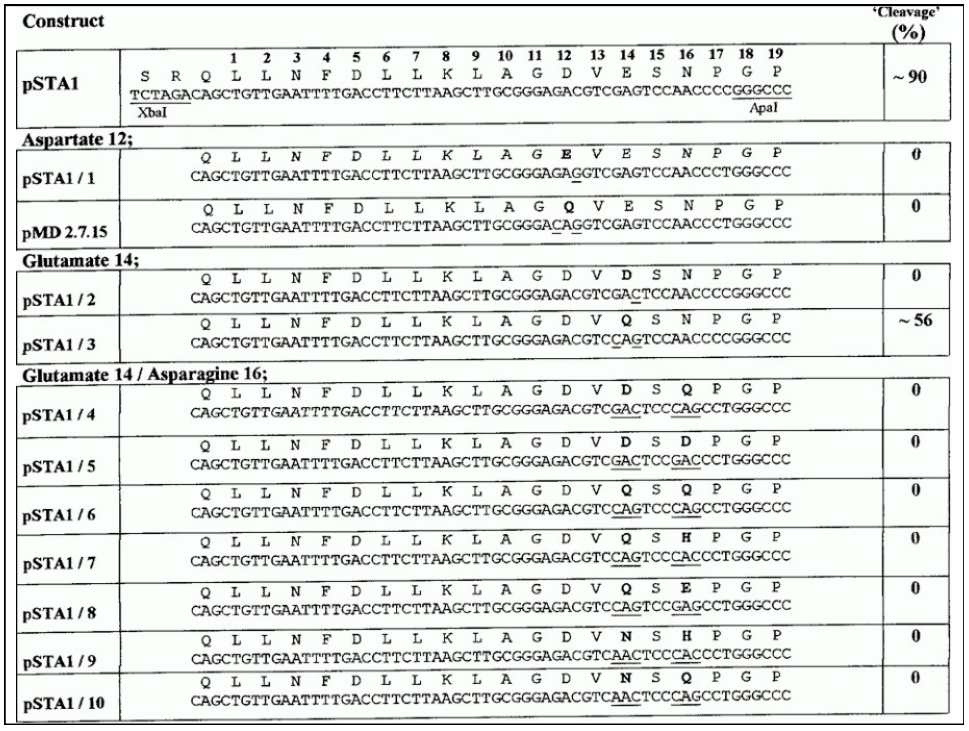


Fig. 5. Scheme of 2A activity. The stage following the addition of the ultimate residue of 2A is shown (step i). Peptidyl(2A)-tRNA is translocated from the A to P site (step ii), allowing the ingress of prolyl-tRNA (step iii). Prolyl-tRNA is unable to attack the peptidyl (2A)-tRNAGly ester linkage, and hydrolysis of the glycyl-tRNA ester bond releases the nascent peptide (steps iv and v). Deacylated tRNA is now present in the P site (mimicking peptide bond formation) and would allow the translocation of prolyl-tRNA (rather than the normal peptidyl-tRNA) from the A to P sites (step vi). Synthesis of the peptide C-terminal of 2A would proceed as normal, although recommencing (rather than 'initiating' sensu stricto) with proline.

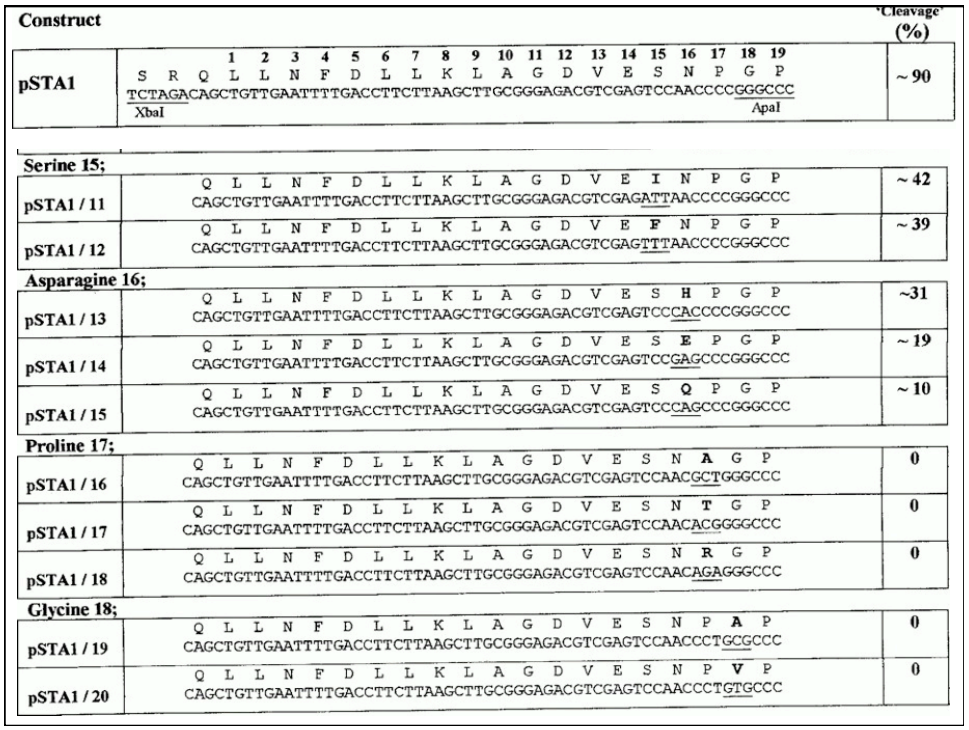
The 'cleavage' activities of foot-and-mouth disease virus 2A site-directed mutants and naturally occurring '2A-like' sequences.

Donnelly ML, Hughes LE, Luke G, Mendoza H, ten Dam E, Gani D, Ryan MD.

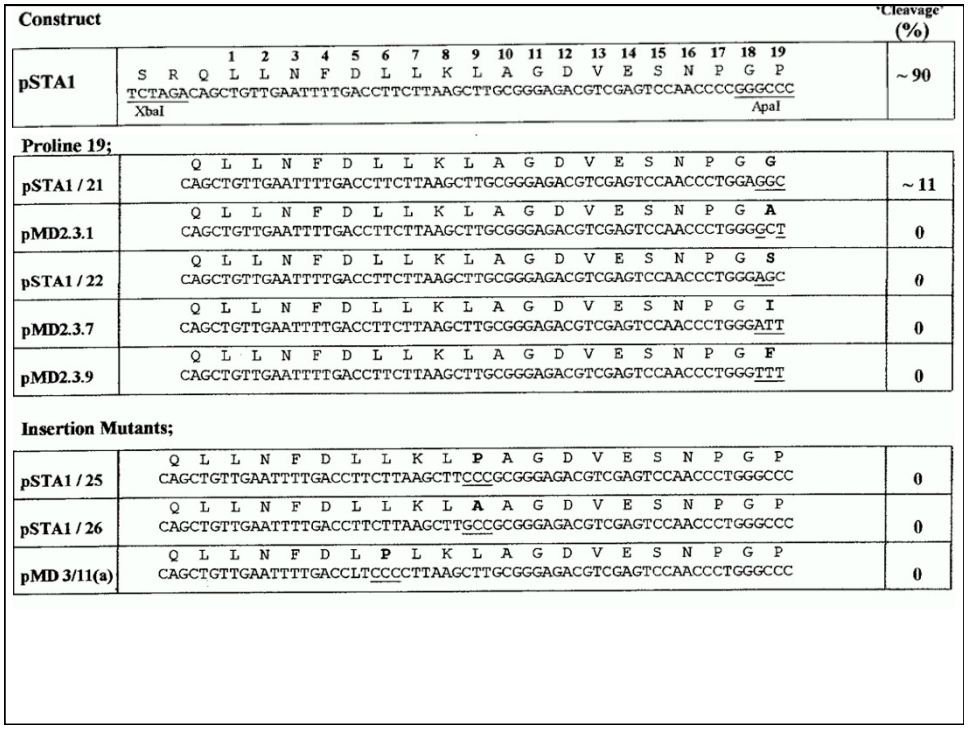
J Gen Virol. 2001 May;82(Pt 5):1027-41.

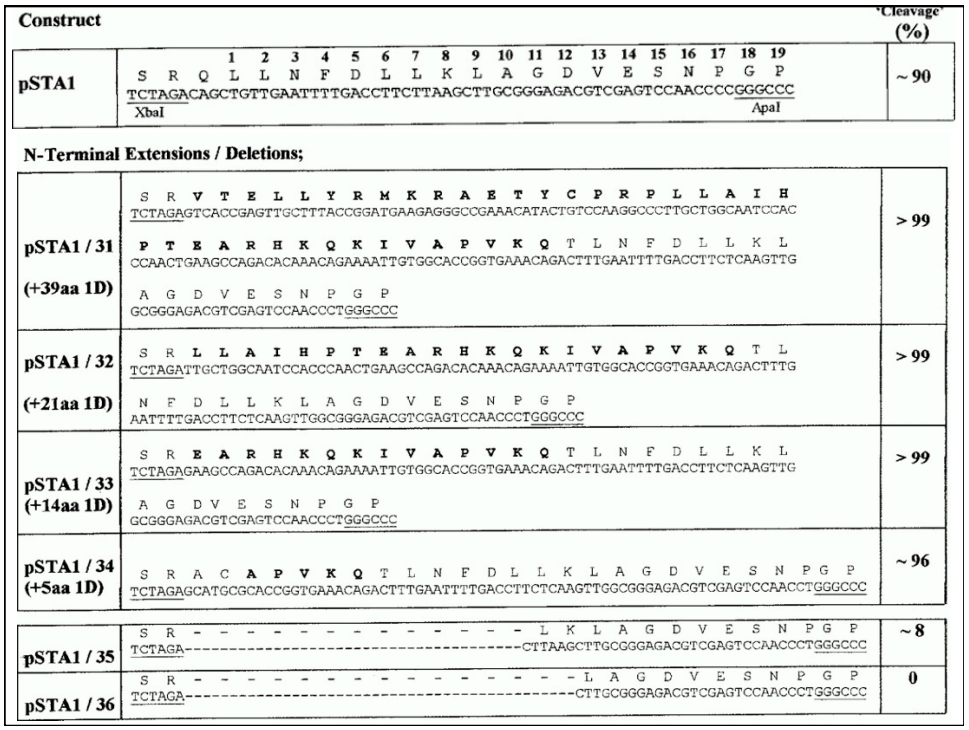


Construct		'Cleavage' (%)
pSTA1	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 S R Q L L N F D L L K L A G D V E S N P G P TCTAGACAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCCC XbaI ApaI	~ 90
Aspartate 12;		
pSTA1 / 1	Q L L N F D L L K L A G **E** V E S N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGAGGTCGAGTCCAACCCTGGGCCC	0
pMD 2.7.15	Q L L N F D L L K L A G **Q** V E S N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGACAGGTCGAGTCCAACCCTGGGCCC	0
Glutamate 14;		
pSTA1 / 2	Q L L N F D L L K L A G D V **D** S N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGACTCCAACCCCGGGCCC	0
pSTA1 / 3	Q L L N F D L L K L A G D V **Q** S N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCCAGTCCAACCCCGGGCCC	~ 56
Glutamate 14 / Asparagine 16;		
pSTA1 / 4	Q L L N F D L L K L A G D V **D** S **Q** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGACTCCCAGCCTGGGCCC	0
pSTA1 / 5	Q L L N F D L L K L A G D V **D** S **D** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGACTCCGACCCTGGGCCC	0
pSTA1 / 6	Q L L N F D L L K L A G D V **Q** S **Q** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCCAGTCCCAGCCTGGGCCC	0
pSTA1 / 7	Q L L N F D L L K L A G D V **Q** S **H** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCCAGTCCCACCCTGGGCCC	0
pSTA1 / 8	Q L L N F D L L K L A G D V **Q** S **E** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCCAGTCCGAGCCTGGGCCC	0
pSTA1 / 9	Q L L N F D L L K L A G D V **N** S **H** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCAACTCCCACCCTGGGCCC	0
pSTA1 / 10	Q L L N F D L L K L A G D V **N** S **Q** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCAACTCCCAGCCTGGGCCC	0



Construct		'Cleavage' (%)
pSTA1	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 S R Q L L N F D L L K L A G D V E S N P G P TCTAGACAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCCC XbaI ApaI	~ 90
Serine 15;		
pSTA1 / 11	Q L L N F D L L K L A G D V E **I** N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGATTAACCCCGGGCCC	~ 42
pSTA1 / 12	Q L L N F D L L K L A G D V E **F** N P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTTTAACCCCGGGCCC	~ 39
Asparagine 16;		
pSTA1 / 13	Q L L N F D L L K L A G D V E S **H** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCCACCCCGGGCCC	~31
pSTA1 / 14	Q L L N F D L L K L A G D V E S **E** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCGAGCCCGGGCCC	~ 19
pSTA1 / 15	Q L L N F D L L K L A G D V E S **Q** P G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCCAGCCCGGGCCC	~ 10
Proline 17;		
pSTA1 / 16	Q L L N F D L L K L A G D V E S N **A** G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACGCTGGGCCC	0
pSTA1 / 17	Q L L N F D L L K L A G D V E S N **T** G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACACGGGGCCC	0
pSTA1 / 18	Q L L N F D L L K L A G D V E S N **R** G P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACAGAGGGCCC	0
Glycine 18;		
pSTA1 / 19	Q L L N F D L L K L A G D V E S N P **A** P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGCGCCC	0
pSTA1 / 20	Q L L N F D L L K L A G D V E S N P **V** P CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGTGCCC	0


Construct
'Cleavage' (%)
pSTA1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
S R Q L L N F D L L K L A G D V E S N P G P
TCTAGACAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCCC
XbaI
ApaI
~ 90
Proline 19;
pSTA1 / 21
Q L L N F D L L K L A G D V E S N P G G
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGAGGC
~ 11
pMD2.3.1
Q L L N F D L L K L A G D V E S N P G A
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGGCT
0
pSTA1 / 22
Q L L N F D L L K L A G D V E S N P G S
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGAGC
0
pMD2.3.7
Q L L N F D L L K L A G D V E S N P G I
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGATT
0
pMD2.3.9
Q L L N F D L L K L A G D V E S N P G F
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGTTT
0
Insertion Mutants;
pSTA1 / 25
Q L L N F D L L K L P A G D V E S N P G P
CAGCTGTTGAATTTTGACCTTCTTAAGCTTCCCGCGGGAGACGTCGAGTCCAACCCTGGGCCC
0
pSTA1 / 26
Q L L N F D L L K L A A G D V E S N P G P
CAGCTGTTGAATTTTGACCTTCTTAAGCTTGCCGCGGGAGACGTCGAGTCCAACCCTGGGCCC
0
pMD 3/11(a)
Q L L N F D L P L K L A G D V E S N P G P
CAGCTGTTGAATTTTGACCLTCCCCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGCCC
0


Construct
'Cleavage' (%)
pSTA1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
S R Q L L N F D L L K L A G D V E S N P G P
TCTAGACAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCCC
XbaI
ApaI
~ 90
N-Terminal Extensions / Deletions;
pSTA1 / 31
(+39aa 1D)
S R V T E L L Y R M K R A E T Y C P R P L L A I H
TCTAGAGTCACCGAGTTGCTTTACCGGATGAAGAGGGCCGAAACATACTGTCCAAGGCCCTTGCTGGCAATCCAC
P T E A R H K Q K I V A P V K Q T L N F D L L K L
CCAACTGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTG
A G D V E S N P G P
GCGGGAGACGTCGAGTCCAACCCTGGGCCC
> 99
pSTA1 / 32
(+21aa 1D)
S R L L A I H P T E A R H K Q K I V A P V K Q T L
TCTAGATTGCTGGCAATCCACCCAACTGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTG
N F D L L K L A G D V E S N P G P
AATTTTGACCTTCTCAAGTTGGCGGGAGACGTCGAGTCCAACCCTGGGCCC
> 99
pSTA1 / 33
(+14aa 1D)
S R E A R H K Q K I V A P V K Q T L N F D L L K L
TCTAGAGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTG
A G D V E S N P G P
GCGGGAGACGTCGAGTCCAACCCTGGGCCC
> 99
pSTA1 / 34
(+5aa 1D)
S R A C A P V K Q T L N F D L L K L A G D V E S N P G P
TCTAGAGCATGCGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGACGTCGAGTCCAACCTGGGCCC
~ 96
pSTA1 / 35
S R - - - - - - - - - - - - - - - L K L A G D V E S N P G P
TCTAGA----------CTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGCCC
~ 8
pSTA1 / 36
S R - - - - - - - - - - - - - - - - - - L A G D V E S N P G P
TCTAGA----------CTTGCGGGAGACGTCGAGTCCAACCCTGGGCCC
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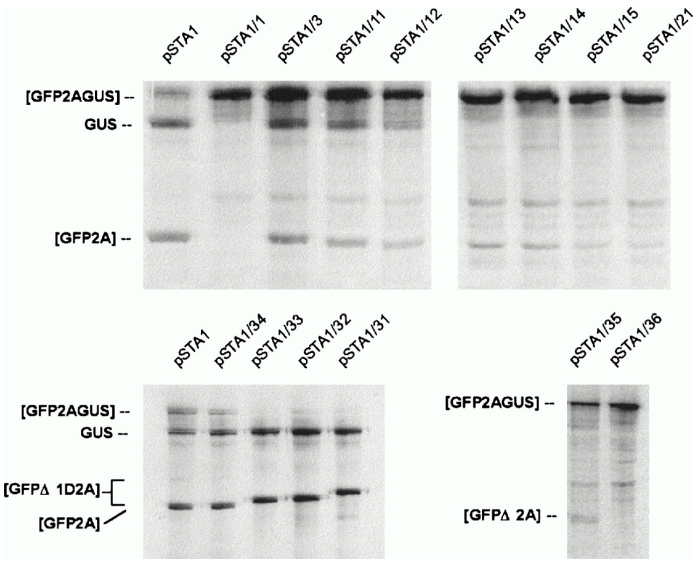


Fig. 1. Translation in vitro. Translation products derived from constructs encoding the wild-type 2A sequence (pSTA1) are shown together with mutated forms. The translation products derived from constructs encoding N-terminally extended forms of 2A (pSTA1/31–34) and deleted forms pSTA1/35,36 are also shown.

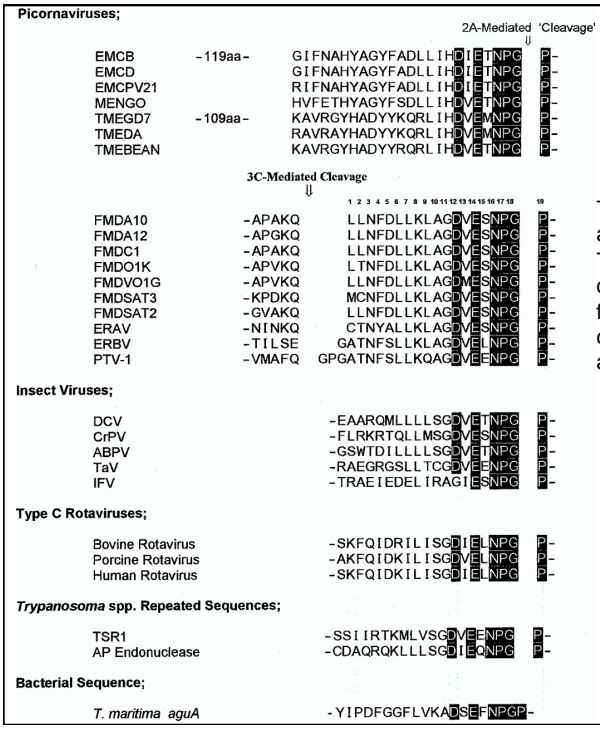


Fig. 2. 2A and 2A-like sequences. The sequences shown are not aligned by computer algorithms. The canonical motif used to probe databases is shown by inverse font. Potential 3C proteinase cleavage sites are shown for the aphtho-, erbo- and teschoviruses.

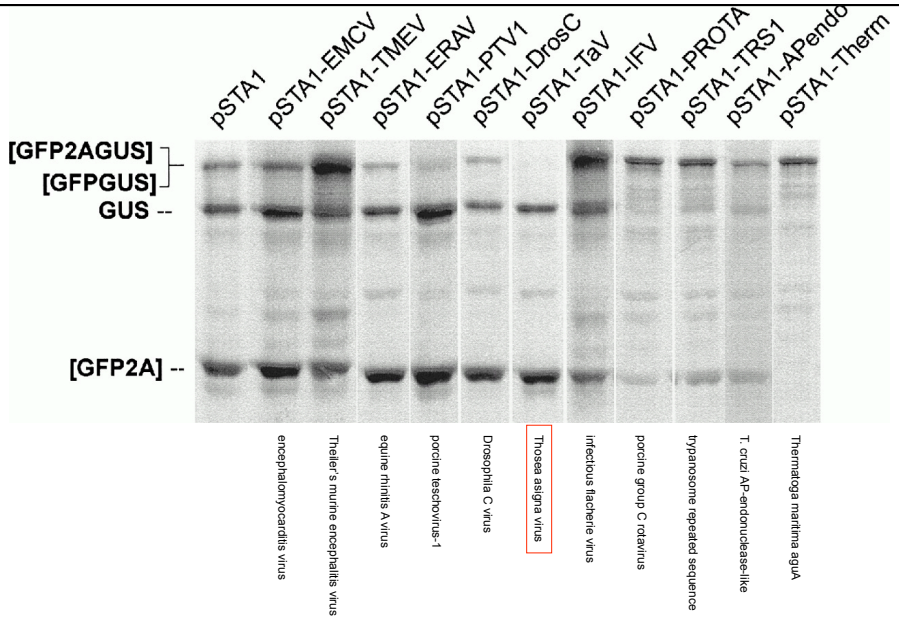


Fig. 3. Translation in vitro. Coupled transcription/translation wheat germ extracts were programmed with the plasmid constructs indicated. Full-length ('uncleaved') and 'cleavage' products are indicated.

Construct	Activity (%)	
pSTA1	~ 90	Q L L N F D L L K L A G D V E S N P G P TCTAGACAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCTGGGCCC XbaI ApaI
pSTA1-TMEV	~ 65	Y H A D Y Y K Q R L I H D V E M N P G P 5′-CTAGATACCATGCTGACTACTACAAACAGAGACTCATACATGATGTAGAAATGAACCCCGGGCC-3′ 3′- TATGGTACGACTGATGATGTTTGTCTCTGAGTATGTACTACATCTTTACTTGGGGC -5′
pSTA1-EMCV	~ 91	H Y A G Y F A D L L I H D I E T N P G P 5′-CTAGACACTACGCTGGTTACTTTGCGGACCTACTGATTCATGACATTGAGACAAATCCCGGGCC-3′ 3′- TGTGATGCGACCAATGAAACGCCTGGATGACTAAGTACTGTAACTCTGTTTAGGTC -5′
pSTA1-ERAV	~ 99	Q C T N Y A L L K L A G D V E S N P G P 5′-CTAGACAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAACCCCGGGCC-3′ 3′- TGTCACATGATTAATACGAGAGAACTTTAACCGACCTCTACAACTCTCGTTGGGGC -5′
pSTA1-PTV1	~ 94	A T N F S L L K Q A G D V E E N P G P 5′- CTAGAGCCACGAACTTCTCTCTGTTAAAGCAAGCAGGAGATGTTGAAGAAAACCCCGGGCC-3′ 3′- TCGGTGCAAGAAGAGAGACAATTTCGTTCGTCCTCTACAACTTCTTTTGGGGC -5′
pSTA1-DrosC	~ 95	A A R Q M L L L L S G D V E T N P G P 5′- CTAGAGCTGCACGTCAGATGTTGCTCTTGTTATCAGGAGATGTTGAGACTAACCCTGGGCC-3′ 3′- TCGACGTGCAGTCTACAACGAGAACAATAGTCCTCTACAACTCTGATTGGGAC -5′
pSTA1-TaV	> 99	R A E G R G S L L T C G D V E E N P G P 5′-CTAGAAGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCC-3′ 3′- TTCTCGGCTCCCGTCCCCTTCAGAAGATTGTACGCCCCTGCACCTCCTTTTAGGGC -5′
pSTA1-IFV	~ 63	T R A E I E D E L I R A G I E S N P G P 5′-CTAGAACGAGGGCGGAGATTGAGGATGAATTGATTCGTCGAGGAATTGAATCAAATCCTGGGCC-3′ 3′- TTGCTCCCGCCTCTAACTCCTACTTAACTAAGCAGCTCCTTAACTTAGTTTAGGAC -5′
pSTA1-IFV(D)	Inactive	T R A E I E D E L I R A D I E S N P G P 5′-CTAGAACGAGGGCGGAGATTGAGGATGAATTGATTCGTCGAGACATTGAATCAAATCCTGGGCC-3′ 3′- TTGCTCCCGCCTCTAACTCCTACTTAACTAAGCAGCTCTGTAACTTAGTTTAGGAC -5′
pSTA1-P.Rota	~ 31	A K F Q I D K I L I S G D V E L N P G P 5′-CTAGAGCTAAATTCCAAATCGATAAAATTTTAATTTCTGGAGACGTCGAATTGAATCCTGGGCC-3′ 3′- TCGATTTAAGGTTTAGCTATTTTAAAATTAAAGACCTCTGCAGCTTAACTTAGGAC -5′
pSTA1-TSR1	~ 18	S S I I R T K M L V S G D V E E N P G P 5′-CTAGAAGCAGTATCATCCGCACTAAGATGCTGGTGTCCGGTGATGTGGAAGAGAATCCCGGGCC-3′ 3′- TTCGTCATAGTAGGCGTGATTCTACGACCACAGGCCACTACACCTTCTCTTAGGGC -5′
pSTA1-APendo	~ 69	C D A Q R Q K L L L S G D I E Q N P G P 5′-CTAGATGTGACGCGCAACGACAAAAGCTACTGCTAAGCGGAGACATTGAGCAGAACCCAGGGCC-3′ 3′- TACACTGCGCGTTGCTGTTTTCGATGACGATTCGCCTCTGTAACTCGTCTTGGGTC -5′
pSTA1-Therm	Inactive	Y I P D F G G F L V K A D S E F N P G P 5′-CTAGATATATTCCAGATTTTGGAGGATTTCTTGTCAAAGCCGATTCTGAGTTCAATCCTGGGCC-3′ 3′- TATATAAGGTCTAAAACCTCCTAAAGAACAGTTTCGGCTAAGACTCAAGTTAGGAC -5′

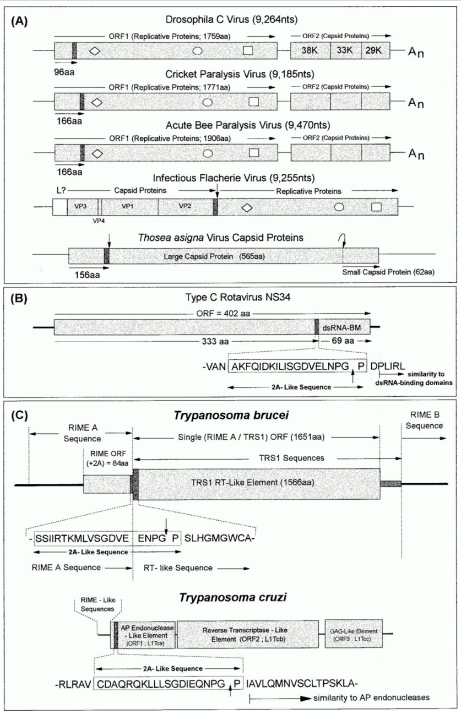


Fig. 4. Occurrence of 2A-like sequences. The positions of 2A-like sequences (dark rectangles) within insect virus polyproteins are shown. The positions of NTP-binding (picornavirus 2C-like), proteinase and RNA polymerase motifs are shown by open diamonds, circles and squares, respectively (A). The position and sequence of the porcine type C rotavirus 2A-like sequence are shown together with the predicted cleavage site and sizes of the 'cleavage' products (B). The positions and sequences of the T. brucei TRS1 and T. cruzi APendo 2A-like sequences are shown together with the predicted cleavage sites (C).

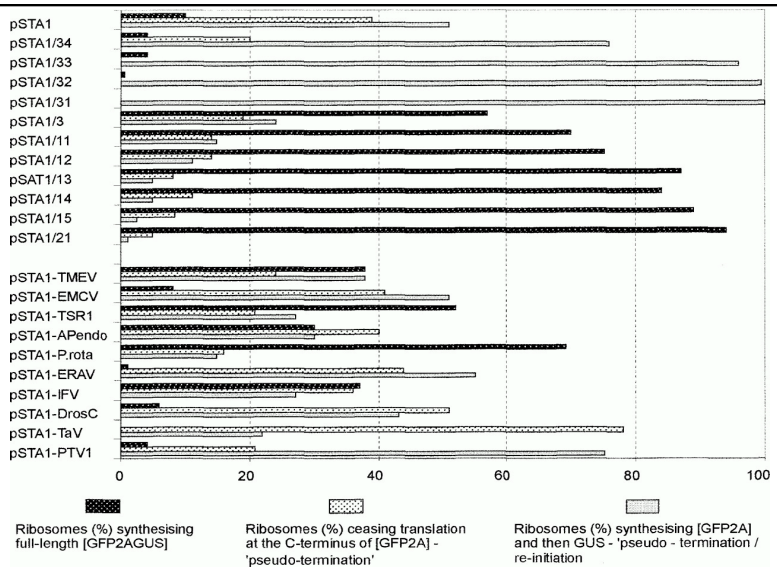


Fig. 5. Translational outcomes. The molar ratios of the translation products [GFP2AGUS], [GFP2A] and GUS were determined by phosphorimaging and used to calculate the proportion of ribosomes which would account for these ratios by either (i) the synthesis of the full-length product, (ii) ceasing translation at the 2A sequence or (iii) synthesizing [GFP2A], hydrolysis occurring to release [GFP2A], and then subsequently synthesizing GUS as a discrete translation product.

Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector.

Szymczak AL, Workman CJ, Wang Y, Vignali KM, Dilioglou S, Vanin EF, Vignali DA.

Nat Biotechnol. 2004 May;22(5):589-94. Epub 2004 Apr 4.
Erratum in: Nat Biotechnol. 2004 Dec;22(12):1590. Nat Biotechnol. 2004 Jun;22(6):760.

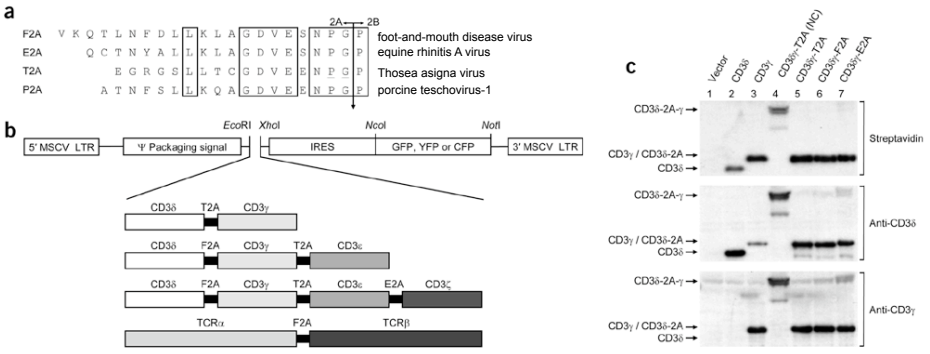
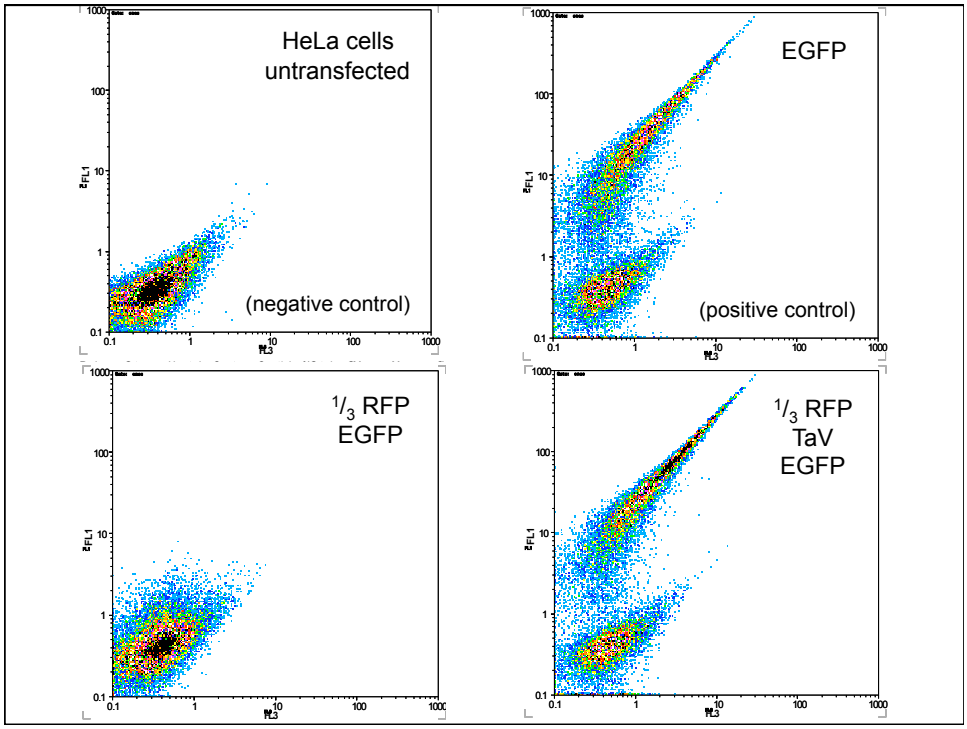


Figure 1. Construction and processing of 2A-linked TCR:CD3 constructs.
(a) Amino acid sequence of the 2A regions of foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), Thosea asigna virus (T2A) and porcine teschovirus-1 (P2A; used later in FRET analysis). Conserved residues are boxed. Underlined amino acids were mutated to alanine for generation of a noncleaving construct. The cleavage point between the 2A and 2B peptides, and thus the N- and C-terminal cistrons, is indicated by the arrow. (b) Schematic of the retroviral vector and 2A-linked TCR:CD3 constructs used. The MSCV long terminal repeat promoter is used to express the 2A-linked construct. The IRES is used to direct translation of GFP. Key restriction sites used for cloning are noted. (c) In vitro transcription/translation of single or 2A-linked CD3 chains. Proteins were translated in the presence of biotinylated lysine and detected by western blot analysis with streptavidin (top). Identity of translation products was confirmed by western blot analysis with rabbit anti-CD3 (middle) or anti-CD3 (bottom) antisera. Note that the anti-CD3[¥] antisera weakly cross-reacts with CD3[¥]. Migration of translation products was consistent with their predicted molecular weight and charge (CD3, Mr = 19,032/pI = 6.41; CD3, Mr = 20,252/pI = 9.03; CD3-2A, Mr = 20,747/pI = 5.57; CD3-2A-, Mr = 41,078/pI = 7.50). Note that the 2A tag, which remains attached to CD3 (the N-terminal protein), affects protein migration and thus results in the comigration of CD3 and CD3-2A. As these proteins were translated in the absence of endoplasmic reticulum, they have not been processed and migrate differently from the native proteins (see Fig. 2c). Differences in band intensity between CD3 and CD3 are likely due to lysine content (9 and 13, respectively). NC, noncleaving.

Using the 2A sequence at UK

Pierce AJ

MI 615, Spring 2007


HeLa cells
untransfected
(negative control)
EGFP
(positive control)
1/3 RFP
EGFP
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