The temporal resolution of the voltage clamp (4 us) is limited by the rise time of the voltage pulse step applied to the amplifier. The capacitance feedback was reset just before the onset of all signals presented. A small, rising slope in charge transfer records, corresponding to a steady-state current, was subtracted from all records presented. This current probably corresponds to a low rate of pump cycling during which three cytoplasmic Na ions are exchanged for two extracellular Na ions (16). All results were obtained at 37°C. Membrane capacitance was determined as the difference between pipette capacitance with the intact membrane patch and the capacitance after patch rupture with subsequent formation of a hydrocarbon (hexane) meniscus, about 1 μm in thickness, across the pipette orifice. The extracellular (pipette) solution contained 120 mM Na-[2-(*N*-mortholino)ethanesulphonic] acid (MES), 0.5 mM MgCl₂, 15 mM *N*-methylglucamine (NMG)-Cl, 10 mM tetraethylammonium (TEA), 10 mM MES, 5 mM EGTA, and 15 mM Hepes, set to pH 7.0 with additional NMG. For the cytoplasmic solution and for pipette solutions with varied Na concentrations, Na-MES was replaced with NMG-MES.

- W. Stürmer, R. Bühler, H.-J. Apell, P. Läuger, J. Membr. Biol. 121, 163 (1991).
- R. Goldshleger, S. J. D. Karlish, A. Rephaeli, W. D. Stein, J. Physiol. 387, 331 (1987); K. Fendler, E.

Grell, E. Bamberg, FEBS Lett. 224, 83 (1987).

- R. F. Rakowski, J. Gen. Physiol. 101, 117 (1993).
 All Na,K pump charge movements defined in this way were completely absent when 100 μM ouabain was included in the pipette (that is, on the extracellular side). With 120 mM extracellular Na, 1 μM ouabain completely abolished charge movements.
- 12. Nearly identical results were obtained by the subtraction of signals after the removal of cytoplasmic Na. Very similar results were also obtained when high cytoplasmic PO₄ (4 mM) and Mg (4 mM) were applied to induce the E₂ pump conformation, and records taken after the removal of PO₄ were subtracted.
- 13. S. Heyse, I. Wuddel, H.-J. Apell, W. Stürmer, J. Gen. Physiol., in press.
- 14. This process requires that the membrane electrical field falls off along the diffusion pathway of the Na ion (that is, the access channel). The position and profile of the membrane field along such a channel, closed at one end, depend minimally on the unknown physical (dielectric) properties of the membrane protein and the geometry of the pore. It seems safe to assume that if the pore is so narrow that water is structured (or bound) within the pore, then membrane potential will fall off along its length. This relation can be assumed for the E₂P3N state. If the channel is wider and the dielectric properties of the transporter protein are

Crystal Structures at 2.5 Angstrom Resolution of Seryl-tRNA Synthetase Complexed with Two Analogs of Seryl Adenylate

Hassan Belrhali, Anna Yaremchuk, Michael Tukalo, Kjeld Larsen, Carmen Berthet-Colominas, Reuben Leberman, Barbro Beijer, Brian Sproat, Jens Als-Nielsen, Gerhard Grübel, Jean-François Legrand, Mogens Lehmann, Stephen Cusack*

Crystal structures of seryl-tRNA synthetase from *Thermus thermophilus* complexed with two different analogs of seryl adenylate have been determined at 2.5 Å resolution. The first complex is between the enzyme and seryl-hydroxamate–AMP (adenosine monophosphate), produced enzymatically in the crystal from adenosine triphosphate (ATP) and serine hydroxamate, and the second is with a synthetic analog of seryl adenylate (5'-O-[N-(L-seryl)-sulfamoyl]adenosine), which is a strong inhibitor of the enzyme. Both molecules are bound in a similar fashion by a network of hydrogen bond interactions in a deep hydrophilic cleft formed by the antiparallel β sheet and surrounding loops of the synthetase catalytic domain. Four regions in the primary sequence are involved in the interactions, including the motif 2 and 3 regions of class 2 synthetases. Apart from the specific recognition of the serine side chain, the interactions are likely to be similar in all class 2 synthetases.

Aminoacyl-tRNA synthetases specifically attach amino acids to the 3'-adenosine of their cognate tRNAs in a two-step reaction. In the presence of Mg^{2+} and ATP, the enzyme first activates the amino acid to

mann fold for class 1 and an antiparallel β fold for class 2 (3). För class 1 synthetases, crystal structures of complexes with ATP have been described for methionyl-tRNA synthetase (MetRS) (4) and GlnRS (5), with the amino acid alone for TyrRS (6) and with the aminoacyl-adenylate for

form the enzyme-bound aminoacyl-adenyl-

ate intermediate. In the second step, the

amino acid is ligated to the cognate tRNA.

The 20 aminoacyl-tRNA synthetases are

divided into two classes of ten (1, 2). The

two classes are characterized by different

short sequence motifs and have quite differ-

ent catalytic domain topologies, the Ross-

SCIENCE • VOL. 263 • 11 MARCH 1994

more like those of membrane than of water, then the electrical field will be forced out of the waterfilled pore and into a position through its closed end. This relation can be assumed for the E_2P2N state.

- C. A. Vandenberg, *Proc. Natl. Acad. Sci. U.S.A.* 84, 2560 (1987); L. Nowak, P. Bregestovski, P. Ascher, A. Herbet, A. Prochiantz, *Nature* 307, 462 (1984).
- 16. K. H. Lee and R. Blostein, Nature 285, 338 (1980).
- 17. D. W. Hilgemann, K. D. Philipson, D. A. Nicoll, *Biophys. J.* **61**, A390 (1992).
- L. A. Vasilets and W. Schwarz, J. Membr. Biol. 91, 43 (1990).
- 19. R. F. Rakowski, L. A. Vasilets, J. La Tona, W. Schwarz, *ibid.* 121, 177 (1991).
- 20. I thank G. A. Frazier and Texas Instruments for designing and building the data acquisitionpulse generator system used in these experiments; R. Lobdill and Axon Instruments for advice and modifying the patch clamp used; P. Foley for technical assistance; V. V. Golobov for mathematical advice; and D. C. Gadsby, R. A. Levis, M. Weber, S. Matsuoka, and K. D. Philipson for helpful discussions. Supported by grants from the American Heart Association and the National Institutes of Health. This work has been reported in part in abstract form (17).

20 October 1993; accepted 14 January 1994

TyrRS (7) and TrpRS (8). A catalytic pathway for the amino acid activation has been proposed for TyrRS (9) and GlnRS (10). For class 2 synthetases, preliminary results have been given for the ATP binding site (11-13), but no structural information is available for the amino acid binding site.

The crystal structures of seryl-tRNA synthetase from T. thermophilus complexed with two different seryl-adenylate analogs described here reveal the aminoacyl-adenylate binding site and the role of several of the conserved residues in class 2 aminoacyltRNA synthetases. The first complex was obtained by soaking native crystals of the enzyme with ATP and serine hydroxamate (SerHx, ⁺H₃N-HCR-CO-N(H)OH, where R is the serine side chain). SerHx inhibits growth in Escherichia coli and has been described as a competitive inhibitor of the enzyme toward the amino acid (14). The crystallographic data reveal strong difference electron density for a serine hydroxamate-AMP (SerHx-AMP) molecule (Fig. 1C). The enzymatic activation of serine hydroxamate has subsequently been confirmed by biochemical studies (see below). Ueda et al. (15) have described a synthetic alanyl-adenylate analog (5'-O-[N-(L-alanyl)sulfamoyl]adenosine) and showed that it was an inhibitor of alanyl-tRNA synthetase activity. Following this idea, we have synthesized the corresponding seryl compound, 5'-O-[N-(L-seryl)-sulfamoyl]adenosine (Fig. 1B) and have co-crystallized it with T. thermophilus seryl-tRNA synthetase (16). This second structure confirms the location of the seryladenylate binding site and comparisons with the first structure indicate that the two analogs do not have the same length linkage

H. Belrhali, A. Yaremchuk, M. Tukalo, K. Larsen, C. Berthet-Colominas, R. Leberman, S. Cusack, EMBL Grenoble Outstation, c/o ILL, BP 156, 38042 Grenoble Cedex 9, France.

B. Beijer and B. Sproat, Biochemical Instrumentation Programme, EMBL, Meyerhofstrasse 1, Postfach 10.2209, D-69012 Heidelberg, Germany.

J. Als-Nielsen, G. Grübel, J-F. Legrand, M. Lehmann, European Synchrotron Radiation Facility, BP 220, F-38043 Grenoble Cedex, France.

^{*}To whom correspondence should be addressed.

between the phosphate or sulfamoyl group and the amino acid (Fig. 1).

Crystals of native seryl-tRNA synthetase from T. thermophilus, grown as described (16) were soaked for 15 hours in a freshly prepared solution of 10 mM ATP (sodium salt; Boehringer) and 20 mM D,L-serine hydroxamate racemic mixture (Sigma). Diffraction data were measured with a 180mm Mar Research image-plate detector on the wiggler beamline W32 at LURE (17), and the data were integrated with the MOSFLM package (18). The starting model for refinement with X-PLOR (19) was the structure of the native enzyme without ligand (21). The initial R factor of 39.8% was reduced to 26.6% by rigid-body refinement and subsequently to 20.7% by a simulating annealing run, followed by 150 cycles of conjugate gradient energy minimization. A difference map calculated with the program SIGMAA (22) revealed strong additional electron density (>14 σ) in each monomer active site and was easily identifiable as an AMP molecule with additional density beyond the phosphate group. Two AMP molecules were then included in the model and, after further cycles of energy minimization, the new difference map (Fig. 2) permitted the seryl hydroxamate (SerHx)



Fig. 1. Structural formulae of (A) normal seryladenylate (Ser-AMP) and the two analogs Ser-AMS (B) and SerHx-AMP (C).

moiety to be built into one of the crystallographically independent active sites with a linkage of the form adenosine-P α -O-N(H)-CO-CHR-NH3⁺. The geometry of the hydroxamate group was taken from the structure of ammonium oxalohydroxamate (22) with the six atoms O-N(H)-CO-C being coplanar. The final model comprising the synthetase dimer with a single SerHx-AMP, a single AMP and 130 water molecules had an R factor of 17.6% (Table 1).

5'-O-[N-(L-seryl)-sulfamoyl]adenosine was synthesized by a method analogous to that described for the alanyl compound (15) except that 2',3'-O-isopropylidene-5'-O-sulfamoyladenosine was reacted with the N-hydroxysuccinimide ester of t-butoxycarbonyl-L-serine benzyl ether [Boc-Ser(Bzl)-OSul. The product from this reaction was obtained in about 60% yield after purification by silica gel column chromatography with a gradient of ethanol from 5 to 25% in dichloromethane. The protecting groups were then removed in two stages. The isopropylidene and Boc protecting groups were cleaved with trifluoroacetic acid-water (5:2 v/v) as described (23). The remaining benzyl-protected product was purified by chromatography (i) on RP-2 silica and development with water and methanol (3:1 v/v) and (ii) on silica and development with methanol and dichloromethane (1:1 v/v). Finally, the benzyl protecting group of the serine side chain hydroxyl group was removed by hydrogenolysis at atmospheric pressure with 10% palladium on charcoal in methanol and water (1:1 v/v) containing 3% acetic acid. The crude product was purified by chromatography on RP-2 silica and development with methanol and water (1:3 v/v); a final purification was performed by reversed-phase high-performance liquid chromatography on a 4-µm C18 column. The structures of the product and intermediates were confirmed by nuclear magnetic resonance spectroscopy. The inhibition constant (K_i) of Ser-AMS for the *E. coli* enzyme was in the nanomolar range.

Seryl-tRNA synthetase was co-crystallized with 350 μ M Ser-AMS. Diffraction data were measured with a 180 mm Mar Research image-plate detector at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The TROIKA beamline (ID10) was used, which has an undulator insertion device as source and a thin silicon monochromator (24). The model was refined as described (Table 1). The final model had an R factor of 18.5% and consisted of the synthetase dimer, two Ser-AMS, and 178 water molecules.

The mode of binding of the two adenylate analogs within the active site cleft is similar (Fig. 3). A complete network of hydrogen bonds, including a few bridging water molecules, determines the strong and specific binding of the two molecules (Fig. 4). Four zones of the synthetase are involved, each containing residues highly conserved in all class 2 synthetases (Figs. 3 and 5).

The purine ring of the adenosine stacks on Phe²⁷⁵ (motif 2) and, on the opposite side, is in Van der Waals contact with Arg³⁸⁶ (motif 3). Adenosine specificity is determined by hydrogen bond interactions to the ring nitrogens (Fig. 4). The Ile^{183} (8) in TrpRS and Leu²⁶¹ in GlnRS (10) play the same role as Val²⁷² in SerRS in providing specificity for ATP by two main chain interactions. Such interactions are apparently absent in MetRS (4) and TyrRS (6). The ribose ring conformation is C3'-endo. The ribose 2'-hydroxyl interacts with the carbonyl oxygen of Thr346 and a water molecule, whereas the 3'-hydroxyl bonds with Glu^{345} . The latter residue is in B strand A4 (shown green in Fig. 3), and is also conserved in class 2 synthetases (Fig. 5). The absolutely conserved motif 2 resi-



Fig. 2 (left). Omit electron density, contoured at 3σ , for the SerHX-AMP molecule calculated with phases derived from the model at the stage before the serine moiety had been included. **Fig. 3 (right).** Ribbon diagram of the catalytic domain of seryl-tRNA synthetase showing Ser-AMS in the active site. The four zones involved in binding the adenylate are the TXE loop (yellow), motif 2 (blue), β strand A4 (green), and motif 3 (red). The figure was prepared with the program RIBBONS (*2*9).

SCIENCE • VOL. 263 • 11 MARCH 1994

due Arg²⁵⁶, disordered in the absence of substrates, interacts with the phosphate (sulfamoyl-) group. The serine hydroxyl acts as a hydrogen bond donor to Glu²⁷⁹ (motif 2) and an acceptor for Thr³⁸⁰ (motif 3). The amino group is stabilized by hydrogen bonds to Thr²²⁵, Glu²²⁷, and Glu²⁷⁹. The binding sites of the two analogs differ only with respect to certain water molecules in the region of the different linkages and an extra hydrogen bond between the carbonyl group and Thr225 in the SerHx-AMP complex. Superposition of the two adenylate molecules (Fig. 6) shows that the extra bond in the SerHx-AMP molecule [-P-O-N(H)-, instead of -S-N(H)- in Ser-AMS] is accommodated by a small relative shift in the position of the phosphate group leaving the adenosine and serine moieties in the same position.

One unexpected result of these measurements is the observation of servl-tRNA synthetase catalyzed activation of serine hydroxamate with ATP to form a novel adenylate-like molecule, SerHx-AMP. The formation of an intermediate distinct from the normal seryl-adenylate has been confirmed by biochemical studies (25) that show that SerHx-AMP has a higher stability to hydrolysis than Ser-AMP. In addition, ³¹P NMR studies support the identification of the linkage in this molecule as -P-O-N(H)- rather than -P-N(OH)- (26). Surprisingly, the synthetase is able to accommodate the extra bond length in this molecule. Recent results have shown that the SerHx-AMP is a substrate for the second step of aminoacylation reaction (25), that is, transfer to tRNA^{Ser}. These observations reopen the question of the exact mechanism of serine hydroxamate inhibition of growth in E. coli (14).

Apart from the specific recognition of the amino acid side chain, the mode of binding of the adenylate observed here is probably similar in other class 2 synthetases. This follows from the conservation of the interacting residues, which are located in four zones in the synthetase sequence (Fig. 5). The first of these zones is the loop between strand $\beta A2$ and helix H9 which brings, in the case of seryl-tRNA synthetases, residues Thr225 and Glu227 (T-X-E) into a position to interact with the amino function of the amino acid. One or both of these residues (or the conservative alternatives, serine and glutamine) are in these positions in all class 2 synthetases with the exception of the threonyl-tRNA synthetases. In motif 2, interacting residues are also conserved, particularly the arginine and carboxylic acid of the Phe-Arg²⁵⁶-X-Glu peptide before the motif 2 loop, and Phe²⁷⁵ and Glu²⁷⁹ after the loop. Binding of either ATP or the adenylate fixes the sidechain conformations of the Arg $^{256}\mbox{-}X\mbox{-}Glu$ **Table 1.** Crystallographic data on the two adenylate complexes. For the Ser-AMS complex, low resclution data, absent because of detector saturation and a large beam-stop shadow, were measured separately on a second crystal with a laboratory source and the two data sets were merged.

Item	Synthetase + ATP + serine hydroxamate	Synthetase + Ser-AMS (inhibitor)		
Space group	P2,	 P2,		
Wavelength (Å)	0.90 (LURE)	0.79(ESRF)	1.54(EMBL)	
Crystals (N)	3	1	1	
Cell parameters				
a (Å)	85.10	86.42	86.18	
b (Å)	125.21	126.35	126.35	
c (Å)	62.40	62.94	62.77	
β (°)	108.8°	109.0°	109.1°	
Resolution (Å)	2.50	2.55	3.50	
		Combined data		
Data				
Total reflections	170878	217462		
Unique reflections	36920	38589		
Average redundancy	4.6	5.6		
Completeness (%)*	87 (87)	91 (88)		
<i>R</i> merge (%)*	6.6 (16.4)	7.5 (10.9)		
/ > 3σ (%)*	78 (51)	89 (73)		
Model				
Refinement R factor (%)	17.6	18.5		
Rms deviations:				
Bond lengths (Å)	0.016	0.016		
Bond angles (°)	3.3	3.2		
Water molecules	130	178		
Average <i>B</i> factor (Å ²)†	25.4, 30.0	17.1, 19.8		

*Highest resolution. †Main chain, side chain.

peptide, and thus it is the first step toward the stabilization of the motif 2 loop that is involved in tRNA acceptor stem interactions. Thus the presence of the intermediate may favor the correct binding of the tRNA in the active site. In the third region, there is a highly conserved glutamic acid (which is glutamine in some synthetases) in strand $\beta A4$, which binds the 3' hydroxyl of the ribose. The 3'-endo confor-



Fig. 4. Diagram of the polar interactions between the Ser-AMS molecule and the synthetase. Residues belonging to motifs 2 and 3 are indicated. Phe²⁷⁵ is stacked with the purine ring of the adenosine. Distances in angstroms are taken from monomer 2 in the structure.

SCIENCE • VOL. 263 • 11 MARCH 1994

REPORTS

	MOTH 2
B3 LOOP L1 B2 H9	A7 LOOP L2 A6
ββ βββ ααααα	ββββββββββ βββββββββββββββββββββββββββ
SHEC 217 LEHTRPLEEEADTSNYALI PTAEVPLTNL	SHEC 256 LPIKMTAHTPCFRSEAGSYGRDTR - GLIRMHOFDKVEMVOIVR
SerTT 211 · · · VWALAETD · · · · · LYLTGTAEVVLNAL	SerTT 244 LPLRYAGYAPAFRSEAGSFGKDVR · GLMRVHQFHKVEQYVLTE
SerSC 213 · · · LYKVIDGEDEK· · · · · YLIATSEQPISAY	SerSC 267 L PI HYVGYSSCFRREAGSHGKDAW- GVFRVHAFEKIEGFVI I
ThrSC 403 · · · MFTFEVEKET · · · · FGLKPMNCPGHCLM	ThrSC 437 LPWRVADFGVIHRNEFSGALS GLTRVHHFGGDDAHIFC
ThrEC 317 · · · MFTTSSENRE · · · · YCIKPMNCPGHVQI	ThrEC 351 LPLRMAEFGSCHRNEPSGSLHGLMRVRGFIDDDAHIFC
GIYBM 233 MENTQIGPSGLVK GELRPTAQQGIEVN	GYBM 271 LPFAAAQIGNSFHNPEISHSGLLHVHEFIMGEIEHFCD
ProDM 1301 VAWVTKSGDSDLAEPIAVRPTSETVMYPA	ProDM 1340 LPI HENQWNNVVHWEFKUPI PFEHIHEFEWUEGHZFAU
PROEC 93 LLRFVDRGER PFVLGPTHEEVIIDL	
HISEC 51 LFKRAIGEVTDVV EKEMYTFEDHNGDS	HEEC 101 GET HET GENERAL HERD OP AM
HISC 102 I LAGRYGED SKLI INLEDUGGEL	ABEC 58 NYSRATTSORCVRAGGKHNDLENVGYTARHHTFFEMLGNFSFGD
AlaBM 39 ··· LLF······ ANAGMNQFKDVFL	AlaBM 65 QYI RVVNTQKCI RAGGKHNDLDDVGKDVYHHTFFEMNGNWSFGD
	ASCEC 205 GFDRYYOIVKCFRDED······LRADROPEFTOIDVETSFM
ASPECITS DYLVPSHVHRGRPSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	ASSC 313 DFERVYEL GPVFRAEN SNTHRHMTEFTGLDMEMAFE
ASPSC 285 - SVFEVITERGREESES SELTVSGOLNGETY	ASHEC 223 - LSKI YT F G PT F R A E N S N T S R H L A E F WM L E PE V A F A
ASTEC 193 GRVDFDRDFFGRETTEN OF ETVOUGENOET	AsnSC 228 · LSRCWTLSPCFRAEK · · · · · · SDTPRHLSEFWMLEVEMCFV
INFC 221 REFLICTHENALDID MYLRIAPELYLKRL	LYSEC 251 GFERVFEINRNFRNEGISVRHNPEFTMMELYMAYA
UNSC 284 KPELTHHNDIDMDMYMBIAPELFLKQL	LYSSC 314 GLDRVYEI GROFRNEG IDMTHNPEFTTCEFYQAYA
PHEC 159 HPARADHDTEWEDT TRLLRTQTSGVQIR	PheEC 183 PPIRIIAPGRVYRNDYDQTHTPMFHQMEGLIVDT
PheSC 281 HPARDLODTFYIKD KLVLRTHSTAISAR	PheSC 347 KPTRLFSIDRVFRNEAVDATHLAEFHQVEGVLADY
	GAYEC 58 YVQPSRRATDGRYGENPNRLQHYYQFQVVIKPSPDN
	MOTIF 3
A3 A4	MOTIF 3
Α3 Α4 βββββββ ββββββββββββββββββββββββββββ	Α5 Η 1 2 βββββββ αααααααααα
АЗ А4 ββββββββ βββββββββββββββββ SerEC 336 GACKTYDLEVWIPAQNT-YR <u>EI</u> SSCSNVWD	ΜΟΤΙ F 3 Α5 βββββββ αααααααααα See C 385 - · · VHTLNGSGLAVGRTLVAVMENYOQADGRIEVP
A3 A4 ββββββββββββββββββββββββββββ SenEC 336 GACKTYDLEVWIPAONT-YREISSCSNVWD SenTT 326 GKWRQVDIEVYLPSEGR-YRETTHSCSALLD	MOTIF 3 Α5 βββββββ αααααααααα SerEC 385 - · VHTLNGSGLAVGRTLVAVMENYOQADGRIEVP SerTT 374 - · AYTLNNTALATPRILAMLLENHGLODGRVRVP
АЗ А4 В В В В В В В В В В В В В В В В В В В	MOTIF 3 A 5 H 1 2 βββββββ ααααααααααα SefEC 385 - · VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SefTT 374 - · AYTLNNTALATPRILAMLLENHQLQDGRVRVP SefSC 398 - · VHCLNSTLAATORALCCILENYQTEDG-LVVP
A3 A4 βββββββββββββββββββββββββββ SenEC 336 GACKTYDLEVWIPAONT-YREISSCSNVWD SenTT 326 GKWRQVDIEVYLPSEGR-YREITHSCSALLD SenSC 347 AAAKYDLEAWFPYOKE-YKEVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIQLDFQ	MOTIF 3 A5 H12 βββββββ αααααααααα SeeEC 385 VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SeeTT 374 AYTLNNTALATPRILANLLENHGLODGRVRVP SeeSC 398 VHCLNSTLAATORALCCILENYQTEDG-LVVP ThrSC 596 VMIHRAILGSVERMTAILTEHFAG KWP
A3 ββββββββ SerEC 336 GACKTYDLEVWIPAONT-YREISSCSNVWD SerTT 326 GKWRQVDIEVYLPSEGR-YRETHSCSALLD SerSC 347 AAAKKYDLEAWFPYQKE-YKELVSCSNCTD ThrSC 542 GAFYGPKIEFTLYDCLDRAWQCGTVQLDFS ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVQLDFS	MOTIF 3 A5 H12 βββββββ ααααααααααα SeeC 385 ··· VHTLNGSGLAVGRTLVAVMENYOOADGRIEVP SerTT 374 ··· AYTLNNTALATPRILAMLLENHGLODGRVRVP SerSC 398 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VHCINSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VHIHRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 VPVMIHRAILGSMERFIGILTEEFAG····· FFP
A3 A4 ββββββββββββββββββββββββββββββββββ	MOTIF 3 A5 H12 βββββββ αααααααααααα SeeC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 ···AYTLNNTALATPRILAMLLENHQLQDGRVRVP SerSC 398 ···VHCLNSTLAATQRALCCILENYQTEDG·LVVP ThrSC 596 ···VMIHRAILGSWERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSMERFIGILTEEFAG·····FFP GyBM 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP
A3 A4 βββββββ ββββββββ SerEC 336 GACKTYDLEVWIPAONT·YREISSCSNVWD SerTT 326 GKWRQVDIEVYLPSEGR·YRETHSCSALLD SerSC 347 AAAKKYDLEAWFPYOKE·YKELVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIQLDFG ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVQLDFS ProDM 1418 GGDYTTTIEAFIS-ASGRAIQGATSHLGQ ProEC 402 RLLIKRGIEV·····GHIFQLGTKYSEAL	MOTIF 3 A5 H12 βββββββ αααααααααααα SeeC 385 ··· VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SeiTT 374 ··· AYTLNNTALATPRILAMLLENHGLOGGRVRVP SeiSC 398 ··· VHCLNSTLAATQRALCCILENYQTEDG-LVVP ThrSC 596 ··· VMIHRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 VPVMIHRAILGSVERMTAILTEHFAG····· FFP GyBM 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP ProDM 1465 · KYVYQNSVGITT·RTIGVMIMVHADNGG-LVLP ProDM 1465 · KYVYQNSVGITT·RTIGVMIMVHADNGG-LVLP
A3 A4 βββββββ ββββββββ SefC 336 GACKTYDLEVWIPAONT.YREISSCSNVWD SefT 326 GKWRQVDIEVYLPSEGR.YRETHSSCSALLD SefC 347 AAAKKYDLEAWFPYOKE.YKELVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIQLDFG ProDM 1418 GQYTTIEAFIS.ASGRAIQGATSHLGQ ProEC 402 RLLIKRGIEVGHIFQLGTKYSEAL HSEC 258 NORLYRGLDYYNRTVFEWVTNSLGSQ	MOTIF 3 A5 H12 βββββββ ααααααααααααα SeeC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 ···AYTLNNTALATPRILAMLLENHGLQDGRVRVP SerSC 398 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP ThrSC 596 ···VHIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSMERFIGILTEEFAG·····FFP GyBM 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP ProDM 1465 · KYVYQNSWGITT·RTIGVMIMVHADNOG·LVLP ProEC 438 ···LLTMGCYGIGVTRVVAAAIEQNYDERG·IVWP H#FC 299 ··· ATPAVGEAMGIEFLVLIVV
A3 A4 βββββββ ββββββββ SerEC 336 GACKTYDLEVWIPAONT.YREISSCSNVWD SerTT 326 GKWRQVDIEVYLPSEGR.YREISSCSNVWD SerSC 347 AAAKYDLEAWFPYOKE.YREVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIQLDFQ ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProEU 402 RLLIKRGIEV GMEC 452 SAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProEC 402 RLLIKRGIEV HEEC 258 NGRLVRGLDYYNR HSSC 325 DLSLARGLDYYTG	MOTIF 3 A5 H12 βββββββ ααααααααααα SeeC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 ···AYTLNNTALATPRILAMLLENHGLODGRVRVP SerSC 398 ··VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ···VHIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVHIHRAILGSWERMTAILTEHFAG·····KWP ThrEC 506 VPVHIHRAILGSWERMTAILTEHFAG······KWP ThrEC 506 VPVHIHRAILGSWERMTAILTEHFAG······KWP ThrEC 506 VPVHIHRAILGSWERMTAILTEHFAG·······KWP ThrEC 506 VPVHIHRAILGSWERMTAILTEHFAG····································
A3 A4 βββββββ ββββββββ SorEC 336 GACKTYDLEVWIPAONT-YREISSCSNVWD SorT 326 GKWRQVDIEVYLPSEGR-YRETHSCSALLD SorSC 347 AAAKKYDLEAWFPYQKE-YKELVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIOLDFQ ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProEC 402 RLLIKRGIEVGHIFQLGTKYSEAL MECC 258 NORLVRGLDYYNRTVFEWVTNSLGSQ HESC 325 DLSLARGLDYYTGLIYEVGGGSVBLH	MOTIF 3 A5 H12 βββββββ αααααααααααα SeeC 385 ···VHTLNGSGLAVGRTLVAVMENYOOADGRIEVP SerTI 374 ···AYTLNNTALATPRILAMLLENHGLODGRVRVP SerSC 398 ···VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ···VHIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSVERMTAILTEHFAG······KWP ThrEC 506 VPVMIHRAILGSVERMTAILTEHFAG······ FPP GyBM 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP ProDM 1465 ···VYYONSWGITT.RTIGVMIMVHADNOG-LVLP ProEC 438 ···ILTMGCYGIGVTRVVAAAIEQNYDERG·IVWP HSEC 299 ··· ATPAVGFAMGLERLVLLV·ONVNP····EFK· HSSC 394 ··· QIPCVGISFGVERIFSLIKORINS···· NYDI
A3 A4 BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ αααααααααααα SeeC 385 · VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 · AYTLNNTALATPRILAMLLENHQLQDGRVRVP SerC 398 · VHCLNSTLAATQRALCCILENYQTEDG·LVVP ThSC 596 · VMIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSMERFIGILTEEFAG·····FFP Gy8M 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP ProDM 1465 KVYQNSWGITT·RTIGVMIWHADNOG·LVLP Prec 438 · ILTMGCYGIGVTRVVAAAIEQNYDERG·IVWP HisC 299 · ATPAVGFAMGLERLVLLV·QNVNP····EFK HaBC 231 · PKPSVDTGMGLERIAAVL·QHVNS····NYDI AaBM 234 · PTKHIDCGLGELERLVSVI·QNKRA····NYDT
A3 A4 βββββββ ββββββββ SerEC 336 GACKTYDLEVWIPAQNT·YREISSCSNVWD SerT 326 GKWRQVDIEVYLPSEGR·YRETHSCSALLD SerSC 347 AAAKKYDLEAWFPYOKE·YKELVSCSNCTD ThrSC 542 GAFYGPKIDI MISDALRWHOCATIOLDFO ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProDM 1418 GGDYTTTIEAFIS·ASGRAIQGATSHHLGO PreEC 402 RLLIKRGIEV·····GHIFQLGTKYSEAL HEEC 258 NGRLVRGLDYYNR·····VINGYEWTNSLGSO HESC 325 DLSLARGLDYYTG·····LIYEVTSASAPP AspEC 469 AVANAYDM······ VINGYEVGGGSVRIH AspEC 455 KYSNSYDF······ FMNGEEILSGAORIH AspEC 455 KTYAAMDY······ LAPGIGEILGEN	A5 H12 βββββββ αααααααααααααα SorEC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 398 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 398 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 398 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SorEC 398 ···VILNTALATPRILAMLLENYGUENGGLEVP SorEC 398 ···VILNTALATPRILAMLENYQUENGELVVP SorEC 398 ···VILNTALATPRILAMLENYQUENGELEVVP Free Sore 398 ···VILNTALATPRILAMLCENYQUENGELEVVP ThrEC 506 VPVMIHRAILGSWERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSWERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSWERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSWERMTAILTEHFAG·····KWP ProDM 1465 KVVQNSWGITT·RVAAAIEONYDERG·····FP GygM 519 EIIPSVIEPSFGVGRIEVENTWWAAIEONYDERG·····FP ProDM 1465 ····································
A3 A4 ββββββββ βββββββββ SerEC 336 GACKTYDLEVWIPAONT.YREISSCSNVWD SerTT 326 GKWRQVDIEVYLPSEGR.YREITHSSCSALLD SerSC 347 AAAKKYDLEAWFPYOKE.YKELVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIOLDFG ThrC 457 GAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProDM 1418 GDYTTIEAFIS.ASGRAIQGATSHHLGO HSEC 258 NGRLVRGLDYNROHIFQLGTKYSEAL HSEC 255 DLSLARGLDYYTGLIYEVVTSASAPP ASPEC 469 AVANAYDMFMNGEEILSGAQRIH ASPEC 455 KYSNSYDFFMGEEILSGAQRIH ASPEC 375 KTVAAMDVLAPGIGEIIGGSURIH ASPEC 379 DTVGCFDLLVPGMGEIIGGSLRED	A5 H12 βββββββ ααααααααααααα SerEC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 ···AYTLNNTALATPRILAMTLENHGLQDGRVRVP SerSC 398 ···VHCLNSTLAATQRALCCILENYQTEDG·LVVP ThrSC 596 ···VMIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSWERFIGILTEEFAG·····FFP GygM 1465 ···VYQNSWGITT·RTIGVMIMVHADNOG·LVLP ProDM 1465 ···VYQNSWGITT·RTIGVMIMVHADNOG·LVLP ProEC 438 ····LTMGCYGIQVTRVVAAAIEQNYDERG·IVWP HeEC 299 ····································
A3 A4 ββββββββ βββββββββ SerEC 336 GACKTYDLEVWIPAONT.YREISSCSNVWD SerT 326 GKWRQVDIEVYLPSEGR.YRETHSCSALLD SerSC 347 AAAKYDLEAWFPYOKE.YREVSCSNCTD ThrSC 542 GAFYGPKIDIMISDALRRWHQCATIQLDFG ThrEC 457 GAFYGPKIEFTLYDCLDRAWQCGTVOLDFS ProEC 402 RLLIKRGIEV GKURGUTIEAFIS-ASGRAIQGATSHHLGQ ProEC 402 RLLIKRGIEV GKURGUTIEAFIS-ASGRAIQGATSHHLGQ HEEC 258 NGRLVRGLDYNR SASC 325 DLSLARGLDYYTG ASPEC 469 AVANAYDM ASPEC 465 KYSNSYDF ASPC 465 KYSNSYDF LAPGIGEILGGSQREE ASPSC 465 KYSNSYDF LAPGIGEILGGSLREE ASPSC 405 KYSNSYDF LAPGIGEILGGSLREE LYSC 409 EITDRFEF	MOTIF 3 A5 H12 βββββββ αααααααααααα SeeC 385 VHTLNGSGLAVGRTLVAVMENYQOADGRIEVP SerTT 374 ATLNNTALATPRILAMLENHGLODGRVRVP SeeC 398 VHCLNSTLAATORALCCILENYOTEDG-LVVP ThrSC 596 VMIHRAILGSVERMTAILTEHFAG FPGWMIHRAILGSVERMTAILTEHFAG KWP ThrEC 506 VVMIHRAILGSVERMTAILTEHFAG ProDM 1465 KVVQNSKGITT ProDM 1465 KVVQONSKGITT FPG299 ATPAVGFAMGLERLVLV MBEC 299 ATPAVGFAMGLERLVLV HISCC 394 OIPCVGISFGVERIFSLIKORINS HABEC 231 PKPSVDTGMGLERIAVUL AABEC 231 PTKHIDCGLGLERLVSVI ABBM 234 PTKHIDCGLGLERLVSVI ASPEC 523 YGTPPHAGLAFGLDRLT MLLTGTDNIRDVIAFPK ASPEC 523 YGTPPHAGGIGLERLVK
A3 A4 BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ αααααααααααα Soft 385 ··· VHTLNGSGLAVGRTLVAVMENYOOADGRIEVP Soft 385 ··· VHTLNGSGLAVGRTLVAVMENYOOADGRIEVP Soft 374 ··· AYTLNNTALATPRILAMLLENHGLODGRVRVP Soft 398 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP Soft 596 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VHCINSTLAATORALCCILENYOTEDG·LVVP Prob 19 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP Prob 1465 KYVYONSWGITT.RTIGVMIMVHADNOG·LVLP ProEC 438 ·· ILTMGCYGIGVTRVVAAAIEQNYDERG·IVWP HSEC 299 ·· ATPAVGFAMGLERLVLLV·ONVP····EFK· HSSC 394 ·· OIPCVGISFGVERIFSLIKORINS····STT- AaEC 231 ·· PKPSVDTGMGLERIAVL·OHVNS····NYDI AaBM 234 ·· PTKHIDCGLGLERLVSVI·QNKRA····NYDT AspEC 523 YGTPPHAGLAFGLDRLTMLLTGTDNIRDVIAFPK AspSC 517 YGCPPHAGGGIGLERVVMFYLDLKNIRRASFVPR AsrEC 426 YGTVPHSGFGLGFERLIAYVTGVQNVRDVIPFPR
A3 A4 BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ ααααααααααααα SefC 385 · VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 · AYTLNNTALATPRILAMLLENHQLQDGRVRVP SerS 398 · VHCLNSTLAATORALCCILENYQTEDG·LVVP ThSC 596 · VMIHRAILGSVERMTAILTEHFAG·····KWP ThrEC 506 VPVMIHRAILGSWERTIGILTEEFAG·····KWP ProDM 1655 KYVQNSWGITT·RTIGVMIWAANEG7SLP ProDM 1655 KYVQNSWGITTVVAAAIEQNYDERG·LVVP HeEC 299 · ATPAVGFAMGLERLVLV·QNVNP···· EFK· HeSC 394 · OIPCVGISFGVERIFSLIKORINS···· STT- AABM 234 · PTKHIDCGLGLERLVSVI·QNVROS···· NYDI AABM 234 · PTKHIDCGLGLERLVSVI·QNKRA···· NYDT AspEC 523 YGTPPHAGLAFGLDRLTMLLTGTDNIRDVIAFPK AspEC 426 YGTVPHAGGGIGLERVWWFYLDLKNIRRASFVPR AspEC 426 YGTVPHAGGGIGLERFISYLYGNVNRDVIPFPR
A3 A4 BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ ααααααααααααααα SorEC 385 VHTLNGSGLAVGRTLVAVMENYOQADGRIEVP SorET 374 AYTLNNTALATPRILAMLLENHGLODGRVRVP SorEC 385 VHTLNNTALATPRILAMLLENHGLODGRVRVP SorEC 385 VHCLNSTLAATQRALCCILENYQTEDG.LVVP ThrSC 596 VMIHRAILGSVERMTAILTEHFAGKWP ThrEC 506 VPVMIHRAILGSMERFIGILTEFAGFFP GYBM 519 EIIPSVIEPSFGVGRILYCILEHNFRMREG7SLP ProDM 1465 KYVQNSWGITT.RIGVAIEQNUPERG.IVWP HeEC 299 ATPAVGFAMGLERLVLLV.QNVNPEFK. HeEC 291 ATPAVGFAMGLERLVLLV.QNVNPEFK. HeEC 231 PKPSVDTGMGLERIAAVL.QHVNSNYDI AlaBM 234 PTKHIDCGLGLERLVSVI.ONKRANYDT AspEC 523 YGTPPHAGLAFGLDRLTMLLTGTDNIRDVIAFPK AspSC 517 YGCPPHAGGGIGLERVVMFYLDLKNIRASFVPR AshC 426 YGTVPHSGFGLGFERLIAYVTGVQNVRDVIPFPR AshC 426 YGTVPHSGFGLGFERFISYLYGNNIKDAIPFYR LysEC 467 HGLPPTAGLGIGIDRWMMLFTNSHTIRDVILFPA
A3 A4 BBBBBBBB BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ ααααααααααααα SefEC 385 ··· VHTLNGSGLAVGRTLVAVMENYOQADGRIEVP SerTT 374 ··· AYTLNNTALATPRILAMULENHGLODGRVRVP SerSC 398 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VMI HRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 VPVMI HRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 ··· VMI HRAILGSVERMTAILTEHFAG····· KWP ProDM 1465 ··· KYVYONSWGITT·RTIGVMI MVHADNOG·LVLP ProDM 1465 ··· VYONSWGITT·RTIGVMI MVHADNOG·LVLP ProEC 438 ··· ILTMGCYGIGVTRVVAAAIEONYDERG·IVWP HeSC 299 ··· ATPAVGFAMGLERLVLLV·ONVNP···· EFK· HeSC 231 ··· PKPSVDTGMGLERIAVLO·OHVNS···· NYDI AAEC 231 ··· PKPSVDTGMGLERIAVL·OHVNS···· NYDI ABBM 234 ··· PTKHIDCGLGLERLVSVI·ONKRA···· NYDT AspEC 523 YGTPPHAGLAFGLDRLTMLLTGTDNIRDVIAFPK AspC 517 YGCPPHAGGGIGLERVVMFYLDLKNIRRASFVPR AshC 426 YGTVPHSGFGLGFERFISYLYGNNRVIPFPR AshC 451 EGSAPHGGFGLGFERFISYLYGNNRIKDAIPFPR AshC 451 EGSAPHGGFGLGFERFISYLYGNNRIKDAIPFPR AshC 451 EGSAPHGGFGLGFERFISYLYGNNRIKDAIPFPR AshC 451 EGSAPHGGFGLGFERFISYLYGNNRIKDAIPFPR AshC 451 EGSAPHGGFGLGFERFISYLYGNNRIKDAIPFPR
A3 A4 BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ αααααααααααα SeeC 385 ··· VHTLNGSGLAVGRTLVAVMENYQOADGRIEVP SerTT 374 ··· AYTLNNTALATPRILAMULENHGLODGRVRVP SeeC 398 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP SeeC 398 ··· VHCLNSTLAATORALCCILENYOTEDG·LVVP ThrSC 596 ··· VMI HRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 VVMI HRAILGSVERMTAILTEHFAG····· KWP ThrEC 506 ··· VMI HRAILGSVERMTAILYCILEHNFRMREGTSLP ProDM 1465 ··· VYONSWGITT·RTIGVMI MVHADNOG·LVLP ProEC 438 ··· ILTMGCYGIGVTRVVAAAIEQNYDERG·IVWP HSEC 299 ··· ATPAVGFAMGLERLVLV ··· ONVP····· EFK· HSSC 394 ··· OIPCVGISFGVERIFSLIKORINS····· NYDI AABM 234 ··· PKPSVDTGMGLERIAVLORVNS····· NYDI AABM 234 ··· PTKHIDCGLGLERLVSVI·· ONKRA····· NYDI AABM 234 ··· PTKHIDCGLGLERLVSVI·· ONKRA····· NYDI AspEC 523 YGTPPHAGLAFGLDRLTMLLTGTDNIRDVIAFPK AspEC 517 YGCPPHAGGGIGLERVVMFYLDLKNIRRASFVPR AshEC 426 YGTVPHSGFGLGFERLIAYVTGVONVRDVIPFPR AshEC 426 YGTVPHSGFGLGFERTISLYUMFYLDLKNIRRASFVPR AshEC 426 YGTVPHSGFGLGFERTISLYUMFYLDLKNIRALSFVPR AshEC 426 YGTVPHSGFGLGFE
A3 A4 BBBBBBBB BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB	A5 H12 βββββββ αααααααααααα SeeC 385 ···VHTLNGSGLAVGRTLVAVMENYQQADGRIEVP SerTT 374 ···AYTLNNTALATPRILAMLLENHGLQDGRVRVP SeeC 398 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP SeeC 598 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP SeeC 598 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP SeeC 598 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP SeeC 598 ···VHCLNSTLAATORALCCILENYQTEDG·LVVP ThSC 596 ···VHCLNSTLAATORALCCILENYGTEDG·LVVP ThSC 596 ··VHCLNSTLAATORALCCILENYGTEDG·LVVP ThSC 596 ··VHCLNSTLAATORALCCILENYGTEDG·LVVP ThSC 596 ··VHCLNSTLAATORALCOLLENYGTEDG·LVVP ThSC 596 ··VHCLNSTLAATORALCOLLENYGTEDG·LVVP ProbM 1465 ·VHCNSVP ProbM 1465 ·VHCNSWGITTRT <rt< td=""> ProbC 438 ··ILTMGCYGIGVTRVAAAIEQNYDERG·IVP ProEC 438 ··ILTMGCYGIGVTRVAAAIEQNYDP HSC 394 ··OIPCVGISFGVERIFSILKORINS.···NYDI AaBM 234 ··PTKHIDCGLGLERVSVI-QNKRAA····NYDT AaBM 234 ··PTKHIDCGLGLERVVMFYLDLKNIRARASFVPR AssC 451 EGSAPHGGFGLGFERTISYLYGNNYDVIPFPR AssSC 517 YGCPPHAGGGGIGLGFERFISYLYGNNNIKDAIPFYR</rt<>

Fig. 5. Alignment of class 2 synthetases in the four zones involved in adenylate binding showing the high conservation of interacting residues (boxed). The figure is adapted from that in (30). The secondary structure assignments derived from the *E. coli* seryl-tRNA synthetase structure are

indicated. Abbreviations used: SerEC: seryl-tRNA synthetase from *E. coli*, TT: *T. thermophilus*, SC: *Saccharomyces cerevisiae*, HU: human, DM: *Drosophila*, BM: *Bombyx mori*.

mation of the ribose may be a characteristic of the class 2 synthetase ATP-binding site since in class 1 synthetases, the ribose is



Fig. 6. Superposition of the active site conformations of Ser-AMS and SerHx-AMP.

bound in the 2'-endo conformation (4, 7). It should be noted that some of the abovecited residues might modify their interactions when the 3' end of the tRNA is in position for the second step of the aminoacylation reaction.

Serine specificity is guaranteed (i) by two hydrogen bond interactions with the side chain hydroxyl group and (ii) by the size of the binding pocket, which would not allow a larger side chain. The motif 3 residue that takes part in serine recognition, Thr^{380} , is not fully conserved among the seryl-tRNA synthetases; in the *E. coli* enzyme it is Ser³⁹¹, which however preserves the same functionality. In *E. coli* phenylalanyl-tRNA synthetase, the Ala²⁹⁴ in the small subunit, which occupies almost exactly this position in mo-

SCIENCE • VOL. 263 • 11 MARCH 1994

tif 3 (Fig. 5), has been implicated in amino acid specificity for this enzyme (27). In the serine binding site, the only other amino acid that could make similar polar interactions is threonine; glycine and alanine would be unfavorable because of the absence of hydrogen bonding capability, and other amino acids would be too large. Model building suggests that discrimination against threonine depends on the fact that its methyl group would be in an unfavorable hydrophilic environment, within 2.5 Å of the main chain carbonyl-oxygen of Ser³⁴⁸ and the side chain of Asn³⁷⁸.

An explanation of the catalytic mechanism of serine activation requires further information on the initial binding sites and conformations of the ATP, magnesium,

and amino acid. Crystallographic measurements at 3.1 Å resolution have shown the conformation of ATP soaked into native crystals of T. thermophilus seryl-tRNA synthetase and its analog AMPPCP soaked into crystals of the complex with tRNA^{Ser} (13). In both cases the triphosphate moiety of ATP was in an extended conformation with the B-phosphate in a position incompatible with the simultaneous binding of the serine as observed in the adenylate complexes. This observation suggests that the initial conformation of the triphosphate in the presence of magnesium and the amino acid is different. This hypothesis is supported by comparison of the two independent active sites in the SerHx-AMP complex. In one active site, a SerHx-AMP molecule is observed as described above. In the other active site the electron density is less well defined but can be interpreted as being a superposition of ATP in a bent conformation and the adenylate product. Recent data at 2.4 Å resolution of a Mn²⁺-ATP complex with the native synthetase shows the ATP in the bent conformation with the γ -phosphate interacting with the universally conserved Arg³⁸⁶ in motif 3 (28). The Mn^{2+} ion is coordinated by the ATP α - and β -phosphates as well as Glu³⁴⁵ and Ser³⁴⁸, both residues being generally functionally conserved in class 2 synthetases (Fig. 5). This conformation of the ATP, when superimposed on that of the adenylate, is very suggestive of an in-line displacement mechanism for serine activation.

REFERENCES AND NOTES

- 1. G. Eriani, M. Delarue, O. Poch, J. Gangloff, D. Moras, Nature 347, 203 (1990).
- D. Moras, Trends Biochem. Sci. 17, 159 (1992) 2
- 3. S. Cusack, C. Berthet-Colominas, M. Härtlein, N. Nassar, R. Leberman, Nature 347, 249 (1990) S. Brunie, C. Zelwer, J-L. Risler, J. Mol. Biol. 216,
- 411 (1990) M. A. Rould, J. J. Perona, D. Söll, T. A. Steitz, 5.
- Science 246, 1135 (1989). 6 P. Brick and D. M. Blow, J. Mol. Biol. 194, 287
- (1987) 7. P. Brick, T. N. Bhat, D. M. Blow, ibid. 208, 83
- (1989)S. Doublié, thesis, University of North Carolina 8
- (1993). a
- A. Fersht, Biochemistry 26, 8031 (1987).
- J. J. Perona, M. A. Rould, T. A. Steitz, ibid. 32, 10. 8757 (1993). J. Caverelli, B. Rees, M. Ruff, J-C. Thierry, D. 11.
- Moras, Nature 362, 181 (1993). S. Cusack et al., in The Translational Apparatus, 12.
- K. H. Nierhaus et al., Eds. (Plenum, New York, 1993), pp. 1-12. 13
- V. Biou, A. D. Yaremchuk, M. A. Tukalo, S. Cu-sack, *Science* **263**, 1404 (1994). 14. T. Tosa and L. I. Pizer, J. Bacteriol. 106, 966 and
- 972 (1971).
- 15. H. Ueda et al., Biochim. Biophys. Acta 1080, 126 (1991).
- 16 M. B. Garber et al., J. Mol. Biol. 213, 631 (1990). 17. R. Fourme et al., Rev. Sci. Inst. 63, 982 (1992).
- A. G. W. Leslie, Joint CCP4 and ESF-EACBM 18. Newsletter on Protein Crystallography, No. 26

Slow Repair of Pyrimidine Dimers at *p53* Mutation Hotspots in Skin Cancer

Silvia Tornaletti and Gerd P. Pfeifer*

Ultraviolet light has been linked with the development of human skin cancers. Such cancers often exhibit mutations in the p53 tumor suppressor gene. Ligation-mediated polymerase chain reaction was used to analyze at nucleotide resolution the repair of cyclobutane pyrimidine dimers along the p53 gene in ultraviolet-irradiated human fibroblasts. Repair rates at individual nucleotides were highly variable and sequencedependent. Slow repair was seen at seven of eight positions frequently mutated in skin cancer, suggesting that repair efficiency may strongly contribute to the mutation spectrum in a cancer-associated gene.

Strong experimental and epidemiological evidence links ultraviolet (UV) irradiation to the development of human skin cancer (1, 2). Mutations in the p53 tumor suppressor gene have been found in a large percentage of such cancers (3), most being localized in exons 5 through 9, which contain conserved sequence blocks (4). The predominant mutations are $C \rightarrow T$ transitions and $CC \rightarrow TT$ double transitions at dipyrimidine sequences, base alterations specifi-

Beckman Research Institute of the City of Hope, Department of Biology, Duarte, CA 91010, USA

cally induced by UV light (5). These findings have implicated the p53 gene as a critical target in UV-related malignancies.

One important step in the prevention of tumor formation is very likely the efficient removal of DNA lesions by cellular DNA repair enzymes. Repair of UV-induced lesions in mammalian cells has been studied at the level of the gene by Southern (DNA) blot techniques (6). It was found that repair is gene-specific (6) and is most efficient on the transcribed strand of active genes (7) because of transcriptionrepair coupling (8). Here we determine the repair rates of UV-induced cyclobu-

SCIENCE • VOL. 263 • 11 MARCH 1994

(April 1992), Daresbury Laboratory, Warrington WA4 4AD. England

- 19. A. T. Brünger, X-PLOR Version 3.1 (Yale Univ. Press, New Haven, CT, 1993).
- 20. M. Fujinaga, C. Berthet-Colominas, A. D. Yaremchuk, M. A. Tukalo, S. Cusack, J. Mol. Biol. 234, 222 (1993).
- R. J. Read, Acta Crystallogr. A 42, 140 (1986). 21
- A. Sameena Begum, V. K. Jain, S. Ramakumar, C. 22. L. Khetrapal, ibid. C44, 1047 (1988).
- J. Castro-Pichel, M. T. Garcia-Lopez, F. G. Delas 23. Heras, Tetrahedron 43, 383 (1987).
- M. S. Lehmann, J. Als-Nielsen, G. Grübel, J-F. Legrand, Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography, No. 28 (May 1993), Daresbury Laboratory, Warrington WA4 4AD, England.
- K. Larsen and R. Leberman, unpublished re-25 sults.
- 26 K. Larsen and S. Perlogaes, unpublished results. 27. P. Kast and H. Hennecke, J. Mol. Biol. 222, 99 (1991).
- 28. H. Belrhali, A. D. Yaremchuk, M. A. Tukalo, S. Cusack, unpublished results.
- M. Carson, J. Mol. Graphics 5, 103 (1987). 29
- S. Cusack, M. Härtlein, R. Leberman, Nucleic 30. Acids. Res. 19, 3489 (1991); L. Ribas de Pouplana, D. D. Buechter, M. W. Davis, P. Schimmel, Prot. Sci. 2, 2259 (1993) for the revised alignment of AlaRS in motif 2.
- 31. We thank R. Fourme and J.-P. Benoit for assistance with diffraction measurements at LURE and all our colleagues who were involved with the first protein crystallography experiments on the TROIKA beamline at the ESRF. Supported in part by NATO collaborative Research Grant 920692 (A.Y., M.T., S.C.). The coordinates have been deposited in the protein data bank, Brookhaven (1SER R code 4; 1SES seryl hydrox-amate SNP; 1SET 5/O/N seryl).

27 September 1993; accepted 24 January 1994

Table 1. Repair of CPDs in the human p53 gene. Repair rates were measured at mutation hotspots in human skin cancer (M) and at surrounding positions. Each position was analyzed three times; codons 278 and 289 were analyzed twice. Results are average values with variation between experiments being about 10 to 25%. The percentage of CPDs after 24 and 48 hours was calculated from the ratio of the band intensity at those times to the band intensity at 0 hour (20).

DNA strand	Codon	Se- quence (5′→3′)	CPDs (%) re- maining after	
			24 hours	48 hours
Nontran- scribed	151 (M) 177 (M) 191 194 195 196 (M) 278 (M) 289	C [°] CC C [°] CC CC [°] T CT [°] T AT [°] C C [°] CGA T [°] CCT CT [°] C	58 95 26 36 13 67 100 10	37 94 4 25 5 62 12 5
Tran- scribed	243 245 (M) 248 (M) 249 285 286 (M) 287 288 291 292 292 294 (M)	C°CAT GC°C C°CG CT°C TT°C CT°C ATT°C CT°T TT°T CT°C	11 56 30 6 2 23 3 3 29 4 30	5 26 21 3 1 18 3 3 18 5 28

^{*}To whom correspondence should be addressed.