A Euryarchaeal Lysyl–tRNA Synthetase: Resemblance to Class I Synthetases

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The sequencing of euryarchaeal genomes has suggested that the essential protein lysyl–transfer RNA (tRNA) synthetase (LysRS) is absent from such organisms. However, a single 62-kilodalton protein with canonical LysRS activity was purified from *Methanococcus maripaludis*, and the gene that encodes this protein was cloned. The predicted amino acid sequence of *M. maripaludis* LysRS is similar to open reading frames of unassigned function in both *Methanobacterium thermoautotrophicum* and *Methanococcus jannaschii* but is unrelated to canonical LysRS proteins reported in eubacteria, eukaryotes, and the crenarchaeote *Sulfolobus solfataricus*. The presence of amino acid motifs characteristic of the Rossmann dinucleotide-binding domain identifies *M. maripaludis* LysRS as a class I aminoacyl–tRNA synthetase, in contrast to the known examples of this enzyme, which are class II synthetases. These data question the concept that the classification of aminoacyl–tRNA synthetases does not vary throughout living systems.

Lysyl–tRNA synthetase is essential for the translation of lysine codons during protein synthesis. In spite of the necessity for this enzyme in all organisms and the high degree of conservation among aminoacyl–tRNA synthetases (*1*), genes encoding a LysRS homolog have not been found by sequence similarity searches in the genomes of two Archaea, *Methanococcus jannaschii* (*2*) and *Methanobacterium thermoautotrophicum* (*3*). This raises the possibility that LysRS, like the asparaginyl– and glutaminyl–tRNA synthetases (*4*), is not present and that lysyl-tRNA (Lys-tRNA) is synthesized by tRNA-dependent transformation of a misacylated tRNA (*5*). Alternatively, these organisms may contain a LysRS activity encoded by a gene that is sufficiently different from those previously identified to prevent its detection by sequence similarity searches.

To investigate the formation of Lys-tRNA in cell-free extracts prepared from *Methanococcus maripaludis*, we used [^{14}C]lysine and homologous total tRNA as substrates (*6*). Amino acid analysis of the ^{14}C-labeled aminoacyl-tRNA produced in this reaction (*7*) indicated that lysine was directly acylated onto tRNA and therefore that Lys-tRNA was not the product of a tRNA-dependent amino acid transformation. This finding was confirmed by the observation that [^{14}C]Lys-tRNA synthesis was inhibited by only one of the 20 canonical amino acids, lysine (Fig. 1). It is consistent with the presence of LysRS, because Lys-tRNA synthesis via a mischarged tRNA would result in a nonlabeled amino acid other than lysine being able to inhibit lysylation of tRNA.

On the basis of these observations, we sought to purify LysRS from *M. maripaludis*. By standard chromatographic procedures (*8*), we isolated a single 62-kD protein, which we purified to homogeneity as judged by both silver staining after denaturing SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2) and the appearance of a single symmetrical peak during size exclusion chromatography. The LysRS activity eluted as a single discrete fraction during all purification steps, indicating that *M. maripaludis* contains only a single LysRS activity. The enzyme could acylate unfractionated tRNA with lysine to the same extent as the *M. maripaludis* crude extract (Fig. 1), confirming that the purified protein is LysRS. In our kinetic characterization of our LysRS for the aminoacylation of *M. maripaludis* total tRNA with lysine (*9*), we calculated a Michaelis constant (K_m) for lysine of 2.2 μM and a catalytic rate constant (k_{cat}) of 1.6 s^{-1}, values comparable with those of other LysRS proteins (*10*). Investigation of the species specificity of Lys-tRNA formation in cell-free extracts showed reactivity between tRNA and RNA-free protein extracts prepared from *Escherichia coli*, *M. thermoautotrophicum*, and *M. maripaludis* (*11*). The only exception was *M. maripaludis* tRNA, which is not a substrate for the *E. coli* enzyme, suggesting that it may lack 2-thiouridine and its derivatives, which are required for

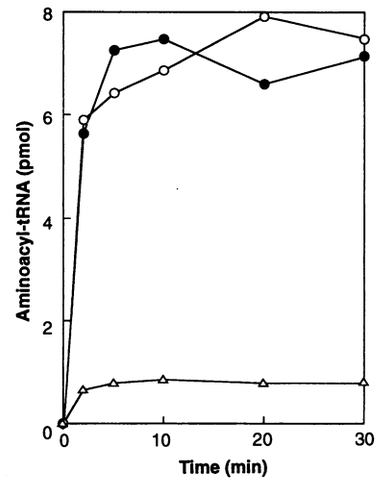

Fig. 1. Direct attachment of lysine to tRNA by *M. maripaludis* protein extracts. Aminoacylation reactions were performed as described in the presence of 200 μg of RNA-free total protein prepared from an S160 extract in a reaction volume of 130 μl. Samples (20 μl) were periodically removed and analyzed. Reactions were performed in the presence of 20 μM [^{14}C]lysine (○), 20 μM [^{14}C]lysine and the 19 canonical amino acids (800 μM labeled with ^{12}C) except for lysine (●), and 20 μM [^{14}C]lysine and 800 μM [^{12}C]lysine (△).

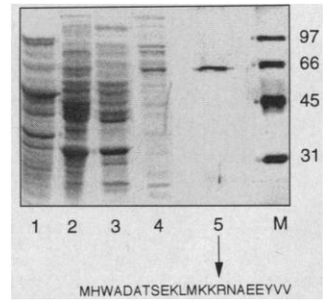

Fig. 2. Purification and NH_2-terminal sequencing of *M. maripaludis* LysRS. Protein purification was monitored by SDS-PAGE: Lane 1: S160 protein extract; lanes 2, 3, 4, and 5: active eluates from Q-sepharose, Mono-Q, Mono-S, and Superose 12, respectively; and lane M: molecular size standards (in kilodaltons). Samples from lane 5 were blotted to polyvinylidene fluoride membranes and subjected to NH_2-terminal sequencing, which yielded the indicated 22–amino acid sequence (*27*). Lanes 1 to 4 were stained with Coomassie blue and lanes 5 and M with silver nitrate.

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the LysRS·tRNA^{Lys} interaction (*12*). These modified nucleotides have previously been detected at position 34 in all known tRNA^{Glu} and tRNA^{Lys} isoacceptors (*13*). In that these modifications are critical for recognition of the tRNA^{Lys} anticodon by LysRS (*12*), their possible absence suggests that tRNA recognition by the M. *maripaludis* enzyme is substantially different from known examples and may explain the inability of an *E. coli* extract to aminoacylate M. *maripaludis* tRNA with lysine.

A portion (22 amino acid residues) (Fig. 2) of the NH_2-terminal sequence of LysRS was determined by protein analysis, and the sequence was used to clone *lysS*, the gene that encodes LysRS. This 22–amino acid sequence is similar to the NH_2-terminus of a predicted protein-coding region (MJ0539) in the M. *jannaschii* genome and one in the M. *thermoautotrophicum* genome, but we find no sequence similarity elsewhere in any available database. The coding sequence MJ0539 has been tentatively identified as a putative aminoacyl–tRNA synthetase (*14*). The NH_2-terminal sequence was used in conjunction with a conserved internal region in the M. *jannaschii* and M. *thermoautotrophicum* open reading frames (ORFs) to clone a 450–base pair (bp) fragment of M. *maripaludis lysS* from genomic DNA by polymerase chain reaction (PCR). This fragment was ^{32}P-labeled and used to isolate a DNA fragment containing the 3′-terminal 1293 bp of the *lysS* gene from a genomic library. The remainder of *lysS* (306 bp) was cloned by PCR from genomic DNA with a 5′ primer based on the NH_2-terminal amino acid sequence of LysRS. After being sequenced, the two portions of *lysS* were used to derive a full-length clone (*15*). The *lysS* gene is 1599 bp long and encodes a 61.3-kD protein, in good agreement with the molecular mass deduced for the native protein from SDS-PAGE (Fig. 3). To confirm the identity of the cloned gene as *lysS*, we subcloned it into pET15b, which allowed the overproduction and subsequent chromatographic purification of M. *maripaludis* LysRS as a hexahistidine (His_6) fusion protein (*16*). This purified His_6-LysRS could aminoacylate with lysine M. *maripaludis* total tRNA to the same extent and at the same rate as the native enzyme (Fig. 4).

The predicted amino acid sequence of M. *maripaludis* LysRS is similar to putative proteins in *Archaeoglobus fulgidus* (*14*), M. *thermoautotrophicum*, and M. *jannaschii* but otherwise appears to have little or no similarity to any known sequences outside of the Euryarchaeota, including that for LysRS from the crenarchaeote *Sulfolobus solfataricus* (a normal class II enzyme). The only exception is a putative ORF in the crenarchaeote *Cenarchaeum* sp., which shares about 28% amino acid identity with the euryarchaeal LysRS protein that we describe, including extensive sequence similarity in the regions containing the HIGH and KMSKS motifs (*17*). Thus, although this protein performs the enzymatic function of a conventional LysRS—the specific esterification of tRNA with lysine—catalysis is accomplished in the context of an amino acid landscape that lacks any sequences corresponding to motifs 1, 2, or 3 (*18*), which are found in known LysRS proteins now classified as class II aminoacyl–tRNA synthetases (Fig. 5). On the basis of extensive similarities in their NH_2-terminal domains, LysRS, aspartyl–tRNA (AspRS), and asparaginyl–tRNA (AsnRS) synthetases were grouped as paralogous enzymes in subclass IIb (*19*). However, the euryarchaeal LysRS bears no similarity to AspRS (the latter is normal), and AsnRS is absent, which leaves AspRS as the only member of this subclass. Thus, there appears to be considerably more evolutionary variation of the aminoacyl–tRNA synthetases than previously thought, a proposal supported by the apparent absence of a recognizable cysteinyl–tRNA synthetase in at least some of the Archaea (*2, 3*) and the existence in the M. *jannaschii* genome of an ORF (MJ1660) encoding an unidentified class II aminoacyl–tRNA synthetase similar to the α subunit of phenylalanyl–tRNA synthetase (*14*).

The euryarchaeal LysRS proteins show variations of the HIGH and KMSKS nucleotide-binding motifs (Fig. 3) characteristic of class I aminoacyl–tRNA synthetases. The occurrence of such sequence motifs has been correlated by structural studies with the topology of the catalytic domain, class I aminoacyl–tRNA synthetases containing a Rossmann fold, and class II aminoacyl–tRNA synthetases containing an antiparallel β sheet. The fact that euryarchaeal

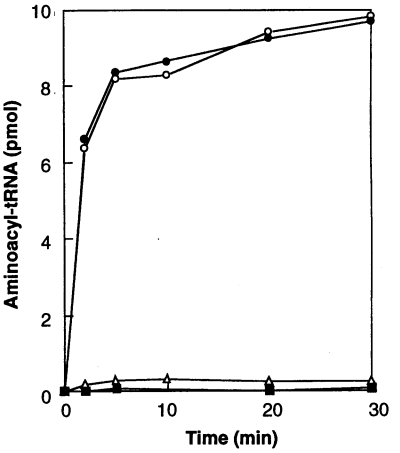

Fig. 4. Aminoacylation of *M. maripaludis* tRNA by purified *M. maripaludis* His_6-LysRS (*16*). Aminoacylation reactions were performed as described (20-μl samples) in the presence of 20 nM enzyme and the following amino acids: 20 μM [^{14}C]lysine (○); 20 μM [^{14}C]lysine and the 19 canonical amino acids (800 μM labeled with ^{12}C) except for lysine (●); and 20 μM [^{14}C]lysine and 800 μM [^{12}C]lysine (△). The squares represent an aminoacylation reaction performed with 20 μM [^{14}C]lysine and 50 nM control protein preparation (His_6–glutamyl-tRNA reductase). The amount of product formation at 30 min indicates that the *M. Maripaludis* total tRNA preparation contains about 32 pmol of tRNA^{Lys} per A_{260} unit, in good agreement with the value for commercial *E. coli* total tRNA reagents (40 pmol per A_{260} unit; Boehringer Mannheim). A_{260}, absorbance at 260 nm.

Fig. 3. Alignment of euryarchaeal LysRS amino acid sequences. The signature sequences characteristic of class I aminoacyl–tRNA synthetases are indicated by underlining. The sequences were aligned with the CLUSTAL W program (*26*). The sequences shown are from *A. fulgidus* (AF), *M. jannaschii* (MJ), *M. maripaludis* (MM), and *M. thermoautotrophicum* (MT).

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MJ    1 LREIMHWADVIAEKLIEERKADKYIVASGITPSGHIHVGNARETLTADAIYKGLINKGVEAELIFIADTYDPLRKLYPFLPKEFEQYIGMPLSEIPCPEGCCESYAEHFL
MM    1 ....MHWADATSEKIMKKRNAEEYVVSSGITPSGHIHIGNARETLTADAVYKGMLKKGAEAKLIFIADDYDPLRKLYPFLPKEFEKYIGMPLSEIPCPQGCCKSYADHFL
AF    1 ....MHWADVIAADLLKRSNSHR..IATGISPSGHIHLGNLREMVTADAIRRALLDAGGEAKIVYIADDFDPLRRRYPFLPEEYENYVGMPLCKIPDPEGCHDSYSEHFL
MT    1 ................LKERDVEEHVVASGTSISGSIHIGNSCDVFIASSIAKSLKKDGFKSRTVWIADDHDPLRKVPYPLPESYEKYLGVPYSMIPCPEGCCESFVEHFQ

MJ  111 RPYLESLDDLGVELTTYRADENYKKGLYDEKIKIALDNREKIMEILNKFRANPLPDDWWPINIVCENCGKL.KTKVIKYDSEKEEITYRCEICGFENTVKPYKGRAKLPW
MM  107 MPFLNSLEDLGVEITTHRANECYKAGMYNEAIITALENRLKIKELLDSYRKEPLADNWYPLNVVCEKCGKMHETKVTSYNSEDKTITYVCK.CGFENTVQPFNGIGKLPW
AF  105 QPFLESLEILGIPVEVRRAYQMYSEGLYENNTRIALKRRDEIARIIAEVTGRELEERWYPFMPLCENCGRINSTRVTSFDENWIYYECDCGHSG....RVGYVGGGKLTW
MT   96 RPFLEALERFRIGVEHYSGARMYTEGLYNDYIRTSLERAPEIREIFNRFRDRPLRDDWLPYNPICEKCGRVNTTEAYDFSGDTVRYRCEC...GFDGEMDIKSGLGKLTW

MJ  220 RVDWPARWSIFNVTIEPMGKDHAAAGGSYDTGVLIAKEIYNYIPPKKVVYEWIQLKVGDKAIPMSSSSKGVVFAVKDWTNIAHPEILRFLLLRSKPTKHIDFDLK.KIPDL
MM  216 RVDWPARWNIFGVTAEPMGKDHAAASGGSYDTGIKIARQIFNYQAPEKMVYEWIQLKIGDKAMPMSSSSGVVFAVKDWTEICHPEVLRFLILKGKPTKHIDFDLK.AISNL
AF  211 RVDWAARWQILSITCEPFGKDHAAAGGSYDTGVRIAREIFDYEPPYPVPYEWIHLKGKG...AMKSSKGIVLPVREMVEVIPPEIVRYITIRVKPERHIEFDPGLGLLDL
MT  203 RVEWAARWKILGVTCEPFGKDHAASGGSYDVSSIISEEIFDYPAPYPVPYEWITLRGE....AMSKSRGVFFTPGQWLEIGPPESLNYFIFRSKPMKHKDFNPDMPFLDL

MJ  329 VDEYDRLEDFYFNNKDK...DELSEEEQEKIRIYELSTPKIPETKPFVIPYRFCSIIAQLTYDEEKEDINMERVFEILRRN.........NYSIDDIDEFSMKKLKDRLLM
MM  325 VDDYDELERKYFELIEKQKTEELNDNENEKISLYELVTPKIPERLPLQVAYRFCSIIAQIALDKETQKIDMERVFDILGRN.........GYNPAEFSEYDKSRLEKRLYM
AF  318 VEEFEE......KFKEKDRSVELS.............LVGEVVYSDVPFRHLIVVGQIA......NWDLEKALEIIERT.........GYTVDDV...TRRDVERRLKY
MT  309 MDQFDRTERIYYGMED.....AASEKEEQKLRNIYRVSMIEEFDLPLRPSYRFMTVACQIAGD......DPERLYDILRRNSQLPEELMDLELDQLTDKQLEQLNERIEN

MJ  428 ARNWALKYGEKLVIISEDEAKEIYEKLKDKQKEWIKYFAEKLKTAEFDALNLHELIYQTAKELGLNPRDAFQASYMILLGKKYGPKLGAFLATLGKDFVIRRYSLFE
MM  427 SKKWASDYGENLEINDFEQAKEQYETLSEEQKAWLKAFSKEVENIEIDANTIHELMYETATKLNLAPKEAFVASYKILLGKNYGPKLGSFLASLKKEFVIGRFNLTE
AF  390 ARKWLEKYAPD..NIKFEIPEKVTAEFSEEEKKFLRAYAERLRS.DMKPEEIHTLVYDVSKEVGIKSSKAFQAIYKAILGKTYGPRVGYFIKSLGVEWVRERIKAAL
MT  408 VKNWLRLYAPEFVKFQVQEELPDVE.LSEPQLKFLQDVADLMESREMSAEELHDEMYSILRRHGLKPQKAFQAIYRVLIGKKMGPRAASFLLSLERDFVIRRLRLEA
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LysRS proteins show the defining motifs of class I aminoacyl–tRNA synthetases forces the unexpected conclusion that the catalytic domains of these enzymes are structurally unrelated to those of their bacterial, eukaryotic, and even certain crenarchaeal counterparts that belong to class II (*20*). Phylogenetic analysis of an overall class I alignment (*21*) did not indicate any unequivocal specific relation between euryarchaeal LysRS and any other class I aminoacyl–tRNA synthetase. Thus, it is unclear whether the euryarchaeal-type LysRS was present in the last common ancestor or arose later through recruitment of another class I enzyme within the Archaea. This uncertainty is surprising because LysRS [like other aminoacyl–tRNA synthetases (*21*)] is conserved through evolution both in the other living kingdoms and in certain Crenarchaeota. To date, archaeal LysRS appears to represent the only known example of class switching among aminoacyl–tRNA synthetases and confirms the unexpected evolutionary origin of euryarchaeal LysRS. LysRS has so far been detected in both major branches of the Archaea: four euryarchaeotes (Fig. 4) and two crenarchaeotes, *Cenarchaeum* sp. and *S. solfataricus*. All show the unexpected class I LysRS except for *S. solfataricus*, which, like all other examples, contains a class II LysRS (whether it also contains a class I LysRS is not known). This dichotomy is sufficiently complex and the number of archaeal examples is sufficiently small that speculation concerning LysRS evolution is not warranted at this stage. However, our data do cast doubt on previous evolutionary proposals based on the presumption of constancy of distribution of any given aminoacyl–tRNA synthetase. The observation that archaeal LysRS is a class I aminoacyl–tRNA synthetase also represents a functional demonstration of nonorthologous displacement (*22*) of a gene required for a core process in gene expression. It differs, for example, from archaeal histone-like proteins (*23*) and the recently discovered DNA topoisomerase VI of *Sulfolobus shibatae* (*24*), both of which have sequence similarity to eukaryotic enzymes of known function.

Our data raise several questions concerning evolution, the first being the aminoacyl–tRNA synthetases themselves. If these enzymes are not evolving as orthologs despite the orthology of the translation apparatus [and the genetic code is constant, with exceptions that are relatively recent compared with our findings (*25*)], then we must compare the relation between evolution of the aminoacyl–tRNA synthetases with that of translation in general and the structure of the genetic code in particular. It appears that the aminoacyl–tRNA synthetases had not achieved as settled an evolutionary condition at the time the universal ancestor of all life gave rise to the primary lineages as has been generally assumed. Second, if the existing universal phylogeny is indeed representative of organismal lineages, then our data begin to speak to the general issue of which functions in the cell were firmly established when the universal ancestor gave rise to the primary lineages and which were not.

	Motif 1		Motif 2		Motif 3	
C.longica.	266	GFLEIETPMM	326	FRNEGIDLTHNPEFTTCE	546	GWGMGIDRVTMFL
H.sapiens	262	GFLEIETPMM	322	FRNEGIDLTHNPEFTTCE	550	GWGMGIDRVAMFL
C.elegans	241	GFLEVETPIM	301	FRNEGIDLTHNPEFTTCE	526	GWGMGIDRLSMIL
S.cerev. c	265	KFIEVETPMM	325	FRNEGIDMTHNPEFTTCE	549	GWGCGIDRLAMFL
L.esculen.	276	EFLEVETPSM	336	FRNEGMDLTHSPEFTMCE	558	GLGMGIDRLTMLL
E.coli (s)	202	GFMEVETPMM	262	FRNEGISVRHNPEFTMME	474	GLGIGIDRMVMLF
E.coli (u)	201	GFMEVETPMM	262	FRNEGISVRHNPEFTMME	474	GLGIGIDRMIMLF
H.influen.	198	GFMEVETPML	258	FRNEGVSVRHNPEFTMLE	472	GEGLGIDRLAMLY
A.calcoac.	204	RFMEVETPMM	264	FRNEGVSTRHNPEFTMIE	477	GQGIGIDRLVMIF
B.subtilis	198	GYLEVETPTM	258	FRNEGVSTRHNPEFTMIE	469	GLGIGIDRLVMLL
S.aureus	194	GFLEVETPMM	254	FRNEGVSTRHNPEFTMIE	465	GLGIGIDRLVMLL
Synechocy.	202	GFIEIETPVL	262	FRNEGVSTRHNPEFTSIE	474	GLGIGIDRLVMLL
C.jejuni	192	GFLEVETPMM	252	FRNEGMDLTHNPEFTTIE	463	GQGIGIDRLVMLL
T.thermop.	185	GFLEVETPIL	245	FRNEGIDHNHNPEFTMLE	454	GLGLGIDRLAMLL
M.genital.	188	GFIEVETPTL	248	FRNEGVDTTHNPEFTSIE	459	GLGIGIDRLVMLL
M.pneumon.	187	GFLEVETPIL	247	FRNEGVDTTHNPEFTSIE	458	GLGIGVDRLVMLL
M.ferment.	188	DYLEAETPFL	248	FRNEGYDTTHNPEFTTIE	456	GCGIGIDRLVMLL
M.hominis	188	NYIEVDTPIL	248	FRNEGVDTTHNPEFTSIE	457	GSGMGIDRTIMTL
S.solfata.	187	GFIEVETPIV	247	FRNEDIDVTHNPEFTLLE	458	GLGIGIDRIVMLV
S.cerev. m	245	NFVEVETPIL	304	FRNEGIDSTHNAEFSTLE	543	GFGLGIDRLCMLF
Consensus		**gϕxxϕxxpϕϕ**		**fRxe.....hxxxfxxxe**		**gϕgϕgϕdRϕϕϕϕϕ**
Most similar						
M.jannaschii	295	DWTNIAHPEI	159	FRANPLPDDWWPINIVCE	507	GKKYGPKLGAFLA
M.maripaludis	291	DWTEICHPEV	155	YRKEPLADNWYPLNVVCE	506	GKNYGPKLGSFLA
M.thermoauto.	290	QWLEIGPPES	160	FRDRPLRDDWLPYNPICE	502	GKKMGPRAASFLL
A.fulgidus	283	EMVEVIPPEI	153	VTGRELEERWYPFMPLCE	466	GKTYGPRVGYFIK

Fig. 5. Alignment of motifs 1, 2, and 3 (*19, 20*) from class II LysRS enzymes against the most similar regions in euryarchaeal LysRS enzymes. Class II and euryarchaeal sequences were separately aligned with CLUSTAL W, and the aligned class II motifs (1, 2, or 3) were used individually to search for similar regions in the euryarchaeal enzymes. Conserved residues are shown in blue; those found only in class II LysRS enzymes are shown in red. The class II LysRS sequences shown are from *Cricetulus longicaudatus* (GenBank accession number Z31711), *Homo sapiens* (D31890), *Caenorhabditis elegans* (U41105), *Saccharomyces cerevisiae* cytoplasm (X56259), *Lycopersicon esculentum* (X94451), *E. coli lysS* (U28375), *E. coli lysU* (X16542), *Haemophilus influenzae* (P43825), *Acinetobacter calcoaceticus* (Z46863), *Bacillus subtilis* (P37477), *Staphylococcus aureus* (L36472), *Synechocystis* sp. (D90906), *Campylobacter jejuni* (M63448), *Thermus thermophilus* (P41255), *Mycoplasma genitalium* (P47382), *Mycoplasma pneumoniae* (AE000055), *Mycoplasma fermentans* (U50826), *Mycoplasma hominis* (P46191), *S. solfataricus* (Y08257), and *S. cerevisiae* mitochondria (X57360).

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6. Frozen *M. maripaludis* cells were resuspended in one volume of buffer A [25 mM Hepes (pH 7.2), 1 mM $MgCl_2$, 30 mM NaCl, 5 mM dithiothreitol (DTT), 4 mM 2-mercaptoethanol, and 10% glycerol], sonicated, and centrifuged at 160,000*g* for 3 hours. This extract (S160) was then exchanged into fresh buffer A and applied to a Q-Sepharose Fast Flow column (all chromatography columns were from Pharmacia). The major protein fraction was then eluted with buffer A containing 300 mM NaCl. Under these conditions, RNA remains bound to the column. The protein extract was then concentrated by ammonium sulfate precipitation, resuspended, and exchanged back into buffer A. *Methanococcus maripaludis* and *M. thermoautotrophicum* total tRNA were prepared by standard methods; *E. coli* total tRNA was from Boehringer Mannheim (Mannheim, Germany). Aminoacylation reactions were performed at 37°C in 100 mM Hepes (pH 7.2), 50 mM KCl, 10 mM $MgCl_2$, 5 mM adenosine triphosphate, 5 mM DTT, bovine serum albumin (0.1 mg/ml), 20 μM [^{14}C-U]lysine (317 μCi/μmol; NEN Dupont, Boston, MA), and tRNA (1 mg/ml).
7. For the analysis of the 3′-esterified amino acid, we removed samples from the aminoacylation reaction, and the attached amino acid was recovered as described (*4*). Samples were then applied to cellulose thin-layer chromatography plates, which were developed in a mixture of methanol (six parts), chloroform (six parts), ammonium hydroxide (two parts), and water (one part) [E. von Arx and R. Neher, *J. Chromatogr.* **12**, 329 (1963)]. Amino acids labeled with ^{14}C were visualized by PhosphorImager and internal standards by ninhydrin staining.
8. Frozen cells (20 g) were used to prepare an S160 extract as described (*6*), from which the major protein fraction was then separated by ammonium sulfate precipitation. This fraction was dialyzed and applied to a Q-Sepharose Fast Flow column that was then developed with an NaCl gradient (0 to 300 mM). LysRS-containing fractions were pooled, dialyzed, and then fractionated by anion-exchange chromatography with a Mono-Q column. Active fractions were again pooled and dialyzed, the pH was adjusted from 7.2 to 6, and the fractions were applied to a Mono-S cation-exchange column that was developed with an NaCl gradient (0 to 250 mM). The LysRS-containing samples were pooled and concentrated before they were applied to a Superose 12 gel filtration column [buffer A (pH 7.2)]. The LysRS fractions from this final step were judged to be pure by silver staining after SDS-PAGE. About 0.4 mg of

LysRS was obtained by this procedure.
9. For the determination of kinetic parameters, LysRS was added to a final concentration of 5 nM, and the lysine concentration was varied in the range between 0.2 and 5 times the K_M value. Sampling and quantification were as described [K.-W. Hong *et al.*, *EMBO J.* **15**, 1983 (1996)].
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16. For expression in *E. coli*, *M. maripaludis lysS* was subcloned into pET15b (Invitrogen) and then used to transform the strain BL21 (DE3) [W. F. Studier, A. H. Rosenberg, J. J. Dunn, J. W. Dubendorff, *Methods Enzymol.* **85**, 60 (1990)]. This transformation allowed the production of His_6-LysRS, which was subsequently purified by nickel-affinity chromatography (QIAGEN) followed by gel filtration with a Superose 12 column (*8*).
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21. A phylogenetic tree was constructed by the maximum parsimony method [J. R. Brown and W. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2441 (1995); G. M. Nagel and R. F. Doolittle, *J. Mol. Evol.* **40**, 487 (1995)]. Amino acid sequences for the HIGH and KMSKS motifs and flanking residues were aligned with CLUSTAL W (*26*), and all insertions and deletions were then removed. A maximum-parsimony tree was derived with the programs SEQBOOT, PROTPARS, and CONSENSE from the PHYLIP 3.5c package [J. Felsenstein, Phylogeny Inference Package (Department of Genetics, Univ. of Washington, Seattle, WA, 1993)]. This procedure gave rise to a phylogenetic tree with low replicate frequencies for more than 50% of the nodes.
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27. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
28. We thank D. Smith and Genome Therapeutics Corporation for access to *M. thermoautotrophicum* genome sequence data before publication; the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University for peptide sequencing and oligonucleotide synthesis; J. Deruère, M. Kitabatake, M. Kumar, M. Prætorius-Ibba, and S. Chaturvedi for advice on cloning strategies and providing materials; and A. Pfeifer and W. B. Whitman for advice and encouragement. Supported by a grant from Bristol-Myers Squibb (D.S.). The sequence of the *M. maripaludis lysS* gene has been deposited in GenBank (accession number AF009824).

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Role of Sensory-Evoked NMDA Plateau Potentials in the Initiation of Locomotion

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Reticulospinal (RS) neurons constitute the main descending motor system of lampreys. This study reports on natural conditions whereby *N*-methyl-D-aspartate (NMDA)–mediated plateau potentials were elicited and associated with the onset of locomotion. Reticulospinal neurons responded in a linear fashion to mild skin stimulation. With stronger stimuli, large depolarizing plateaus with spiking activity were elicited and were accompanied by swimming movements. Calcium imaging revealed sustained intracellular calcium rise upon sensory stimulation. Blocking NMDA receptors on RS neurons prevented the plateau potentials as well as the associated rise in intracellular calcium. Thus, the activation of NMDA receptors mediates a switch from sensory-reception mode to a motor command mode in RS neurons.

Locomotion can be initiated, guided, and controlled by an array of sensory cues (*1*). Cutaneous inputs elicit bouts of locomotion in intact lampreys (*2*), but the cellular mechanisms by which this occurs are still unknown and are the subject of this study. We used a semi-intact in vitro preparation to characterize the cellular mechanisms responsible for the transition from sensory responses to motor activity, such as swimming (*3*). The advantage of such a preparation is that the sensory inputs are left intact as well as some of the muscles, so that active behavior may be elicited with all the advantages of a standard in vitro preparation. Reticulospinal (RS) neurons constitute the main descending motor system (*4*). They receive sensory inputs from several modalities (*5*–*7*), including cutaneous inputs (*8*), and in turn they make direct synapses with motoneurons and interneurons involved in the segmental generation of locomotion (*9*). Because of their large size and easy access for electrophysiological studies in controlled in vitro conditions, lamprey RS neurons provide an excellent model for investigation of the mechanisms underlying the transformation of sensory inputs into motor commands in vertebrates.

An in vitro brainstem preparation (*10*) was used in which the skin covering the dorsal head region was left attached (Fig. 1A). Mechanical stimulation of the skin elicited postsynaptic potentials (PSPs) in RS neurons (Fig. 1B). The amplitude of the synaptic responses showed a remarkable linear relation with the stimulus strength when mild stimulation was used (Fig. 1B). Under these conditions, RS cells behaved as close followers of sensory inflow, the time course of the excitatory PSPs being perfectly tuned to the variation of the force applied to the skin (inset of Fig. 1D). Because somatosensory inputs to RS neurons involve a di-synaptic pathway, this close relation implies powerful synaptic connections. When stronger stimuli were delivered to the skin, large depolarizing plateaus were elicited, which were accompanied by spiking activity (Fig. 1C); the stimulus-response relation then switched from a linear proportional function to a nonlinear function (Fig. 1D). In a semi-intact preparation (*11*) in which the brainstem and rostral spinal cord were exposed in vitro with the tail left intact to move freely (Fig. 2A), the depolarizing plateaus were accompanied with swimming movements. A long-lasting de-

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