8. The second ATG is in a more favorable environment with eight out of nine positions (CCACCATGC) matching the Kozak consensus sequence (CC)ACCATGG [M. Kozak, *Cell* **44**, 283 (1986)] as compared to a match at six of the nine positions (GGTCCATGG) for the more 5′ ATG. 5′ rapid amplification of cDNA ends [M. A. Frohman, M. K. Dush, G. R. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8998 (1988)] performed on 1 μg of APL and HL60 total RNA suggested that we were lacking less than 50 to 100 bp of 5′ sequence. Also, our estimate of the size of the largest, abundant product on Northern blots is 4.5 kb, close to the 4.46 kb contained in the consensus sequence.
9. We used the *PML* product amino acid sequences to search the OWL10.0 protein database [D. Akrigg *et al.*, *Nature* **335**, 745 (1988)] with the program PROSRCH [J. F. Collins, A. F. W. Coulson, A. Lyall, *Comput. Appl. Biosci.* **4**, 67 (1988)] on a distributed array processor.
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19. A cDNA library was constructed by D. Simmons from poly(A)$^+$ selected RNA from a blood sample from individual APL512. The library was screened with both *RARA*- and *PML*-specific probes, and the fusion cDNA clones purified. A second fusion message was isolated from individual APL511 blood by the combination of reverse transcription from a *RARA*-specific primer and polymerase chain reaction amplification with both *PML* and *RARA* primers, followed with subcloning in pBluescript (Stratagene). Reverse transcription of APL total RNA and subsequent PCR amplification were performed as described in J. Borrow *et al.* (in preparation).
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21. The translocation breakpoint in the variant APL translocation was detected with the *PML* cDNA probe pAGU3. No abnormalities were observed in *RARA* when we used the genomic probes LCN4A3/A, /B, and /C (*2*) on Bam HI and Eco RI digests. LCN4A3/D was also used, which detects the Bam HI fragment separating LCN4A3/A and /C, and p124/BE1.4, which hybridizes to the Eco RI and Bam HI fragments at the 5′ end of *RARA* (*2*). These five genomic probes cover the entire region of *RARA* in which APL translocations have been seen to occur.

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26. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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The N-End Rule in Bacteria

JOHN W. TOBIAS, THOMAS E. SHRADER, GABRIELLE ROCAP, ALEXANDER VARSHAVSKY

The N-end rule relates the in vivo half-life of a protein to the identity of its amino-terminal residue. Distinct versions of the N-end rule operate in all eukaryotes examined. It is shown that the bacterium *Escherichia coli* also has the N-end rule pathway. Amino-terminal arginine, lysine, leucine, phenylalanine, tyrosine, and tryptophan confer 2-minute half-lives on a test protein; the other amino-terminal residues confer greater than 10-hour half-lives on the same protein. Amino-terminal arginine and lysine are secondary destabilizing residues in *E. coli* because their activity depends on their conjugation to the primary destabilizing residues leucine or phenylalanine by leucine, phenylalanine–transfer RNA–protein transferase. The adenosine triphosphate–dependent protease Clp (Ti) is required for the degradation of N-end rule substrates in *E. coli*.

AMONG THE FUNCTIONS OF PROtein degradation in living cells are the elimination of abnormal proteins and the temporal control of many cellular processes that involve short-lived regulators (*1*). The metabolic instability of a regulatory protein allows for rapid adjustment of its intracellular concentration through changes in the rate of its synthesis or degradation. Features of a protein that confer metabolic instability are called degradation signals. An essential component of one degradation signal is the protein's N-terminal residue (*2*). The presence of this signal, named the N-degron (*3*), is manifested as the N-end rule, which relates the metabolic stability of a protein to the identity of its N-terminal residue. Distinct versions of the N-end rule operate in all eukaryotes examined, from yeast to mammals (*2–8*). The eukaryotic N-degron comprises two distinct determinants: a destabilizing N-terminal residue and a specific internal lysine residue (or residues) (*5, 7*). The latter is the site of formation of a substrate-attached multiubiquitin chain that is required for degradation of the substrate (*7*).

The N-end rule is organized hierarchically: N-terminal Asp and Glu (and Cys in mammalian reticulocytes) are secondary destabilizing residues in that they are destabilizing through their conjugation, by Arg-tRNA–protein transferase, to Arg, one of the primary destabilizing residues (*2, 6, 8, 9*). N-terminal Asn and Gln are tertiary destabilizing residues in that they are destabilizing through their conversion, by a specific deamidase, into the secondary destabilizing residues Asp and Glu (*6*).

In eukaryotes, linear ubiquitin (Ub) fusions to a test protein such as β-galactosidase (βgal) are deubiquitinated by Ub-specific processing proteases irrespective of the identity of the residue at the Ub-βgal junction (*2*). This finding has made it possible to expose in vivo different residues at the N-termini of otherwise identical test proteins. The Ub fusion technique has so far been inapplicable in bacteria, which lack Ub and Ub-specific enzymes (*10*). The isolation of *UBP1*, a gene for a Ub-specific processing protease of *Saccharomyces cerevisiae* (*10*), has allowed us to bypass this difficulty and to test for the presence of the N-end rule in bacteria.

When expressed in *E. coli* (*11*), the yeast Ubp1 protease efficiently deubiquitinated Ub–X-βgals (X is the junctional residue) both in *E. coli* extracts (*10*) and in vivo (Fig. 1). As expected (*2, 10*), Ub–X-βgals retained their N-terminal Ub moieties in con-

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

genic *E. coli* that lacked Ubp1 (Fig. 1B). Pulse-chase experiments with Ub–X-βgals in *UBP1*-expressing *E. coli* (*12*) showed that the Ubp1-produced X-βgal proteins were either long-lived or metabolically unstable, depending on the identity of their N-terminal residue X (Figs. 1A and 2). These measurements, carried out with 19 X-βgals, yielded the N-end rule in *E. coli* (Table 1). Whereas N-terminal Asn and Gln are tertiary destabilizing residues in eukaryotes and Asp and Glu are secondary destabilizing residues in eukaryotes, they are all stabilizing in *E. coli* (Table 1).

Leu, Phe–tRNA–protein transferase (L/F–transferase), which is present in Gram-negative bacteria but absent from eukaryotes, catalyzes the conjugation of Leu or Phe to sterically accessible N-terminal Arg or Lys in proteins or peptides (*13*). Although this enzyme was described years ago (*13*), its function has remained obscure. An *E. coli* mutant lacking L/F-transferase activity was isolated by Soffer and Savage (*14*). We used this *aat*$^-$ (amino acid transferase) strain, as well as an independently constructed *aat*$^-$ mutant (*15*), to determine whether the in vivo destabilizing property of N-terminal Arg or Lys (Table 1) is dependent on the presence of L/F–transferase. Pulse-chase analyses with normally short-lived X-βgals in wild-type (wt) and congenic *aat*$^-$ strains (Figs. 1A and 2 and Table 1) showed that Arg-βgal and Lys-βgal are long-lived proteins in the absence of L/F–transferase. Thus, *E. coli* L/F–transferase is a component of the N-end rule pathway that is essential for the degradation of substrates bearing N-terminal Arg or Lys but is not required for the degradation of substrates bearing any of the other primary N-terminal destabilizing residues (Phe, Leu, Tyr, and Trp) (Table 1).

L/F–transferase catalyzes in vitro the conjugation of Met (at much lower rates than

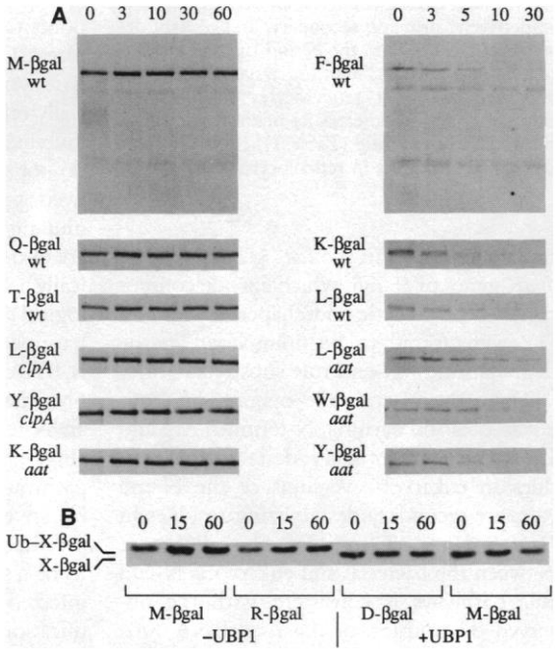

Fig. 1. Metabolic stability of X-βgal in *E. coli* is a function of its N-terminal residue. (**A**) *Escherichia coli* expressing the yeast Ubp1 protease and Ub-X-βgal proteins (*11*) were labeled with ^{35}S, followed by a chase, protein extraction, immunoprecipitation, and electrophoretic analysis of X-βgals (*12*). Chase times (in minutes) are indicated above the lanes. N-terminal residues of X-βgals are indicated by the following single-letter abbreviations for amino acids: 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. To show the entire electrophoretic patterns of representative βgal immunoprecipitates, we present the patterns from Met-βgal and Phe-βgal above the cropped images of analogous patterns for other X-βgals. The designations wt, *aat*, and *clpA* indicate the relevant genetic backgrounds (*15*) of *E. coli* strains used in the pulse-chase assay (*12*) with an indicated X-βgal. (**B**) Pulse-chase analysis was carried out as in (A) with *E. coli* that expressed Ub–Met-βgal, Ub–Arg-βgal, Ub–Asp-βgal, or Ub–Met-βgal either in the presence or absence of Ubp1, as indicated.

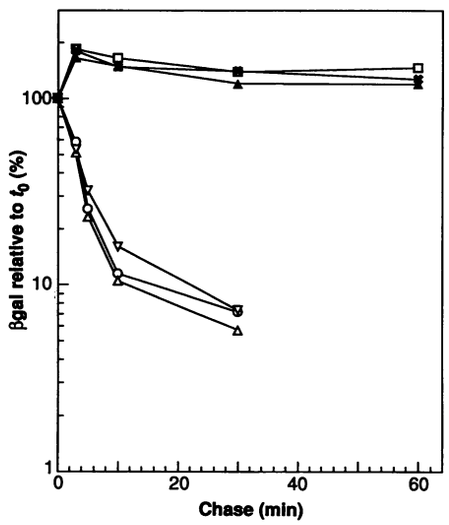

Fig. 2. Kinetics of X-βgal degradation in *E. coli*. The electrophoretic patterns of X-βgals (Fig. 1) were quantified with phosphor storage technology (*22*). Time zero (t_0) corresponds to the start of a chase. Closed and open triangles and open circles denote, respectively, Met-βgal, Lys-βgal, and Phe-βgal in wt *E. coli* (*12*). Crosses and inverted triangles denote, respectively, Lys-βgal and Phe-βgal in a null *aat*$^-$ mutant (*15*). Squares denote Phe-βgal in a null *clpA*$^-$ mutant (*15*). Analogous decay curves were also determined for all of the other X-βgals in wt *E. coli* and for most X-βgals in the *aat*$^-$ and *clpA*$^-$ mutants (*16*). These curves, which yielded the half-life data of Table 1 (*12*), were indistinguishable from the representative examples shown here. Each decay curve was determined at least twice, in independent experiments, with the results differing by less than 15%. For reasons that are not understood, the in vivo degradation of X-βgals in *E. coli* did not obey first-order kinetics but instead slowed with time. A nonexponential in vivo degradation has also been observed with X-βgals and other N-end rule substrates in yeast (*2, 8*). Because a single half-life cannot be assigned to a protein degraded with non–first order kinetics, we determined "initial" half-lives by assuming first-order kinetics between the chase points used. The residual incorporation of ^{35}S into X-βgals continued for ~3 min after the end of a 2-min pulse at 36°C (at t_0). This ~3-min post-pulse incorporation is clearly seen with the long-lived X-βgals but is largely obscured with the short-lived X-βgals because of their rapid degradation. For the short-lived X-βgals, the half-life values (Table 1) were determined between 3 and 10 min of chase. For the long-lived X-βgals, an approximate lower limit on their half-lives (>10 hours) was determined between 10 and 60 min of chase. A small (less than 3%) but reproducible decrease of ^{35}S in the long-lived X-βgals between 3 and 10 min of chase apparently resulted from transient degradation unrelated to the N-end rule, because it persisted in a *clpA*$^-$ mutant (squares).

Table 1. The N-end rule in *E. coli*. Approximate in vivo half-lives of X-βgal proteins in either wt or *aat*$^-$ *E. coli* strains at 36°C were determined as described (*12*) (Fig. 2). The half-life values for X-βgal in wt and *aat*$^-$ *E. coli* are presented next to the half-lives of X-βgal in the yeast *S. cerevisiae* at 30°C (*2, 5*). A question mark at Pro indicates its unknown status in the *E. coli* N-end rule (a low rate of the Ubp1-mediated deubiquitination of Ub–Pro-βgal in *E. coli* (*10*) has so far precluded pulse-chase experiments with Pro-βgal). All of the X-βgals were found to be long-lived in the *clpA*$^-$ mutant (Figs. 1 and 2) (*16*).

X	Half-life of X-βgal		
	E. coli wt	*E. coli* *aat*$^-$	*S. cerevisiae* wt
Arg	2 min	>10 hours	2 min
Lys	2 min	>10 hours	3 min
Phe	2 min	2 min	3 min
Leu	2 min	2 min	3 min
Trp	2 min	2 min	3 min
Tyr	2 min	2 min	10 min
His	>10 hours	>10 hours	3 min
Ile	>10 hours	>10 hours	30 min
Asp	>10 hours	>10 hours	3 min
Glu	>10 hours	>10 hours	30 min
Asn	>10 hours	>10 hours	3 min
Gln	>10 hours	>10 hours	10 min
Cys	>10 hours	>10 hours	>20 hours
Ala	>10 hours	>10 hours	>20 hours
Ser	>10 hours	>10 hours	>20 hours
Thr	>10 hours	>10 hours	>20 hours
Gly	>10 hours	>10 hours	>20 hours
Val	>10 hours	>10 hours	>20 hours
Pro	?	?	>20 hours
Met	>10 hours	>10 hours	>20 hours

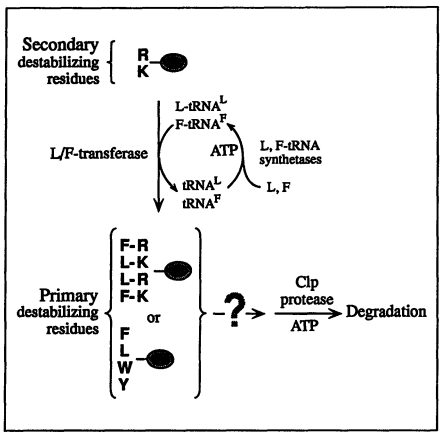

Fig. 3. The N-end rule pathway in *E. coli*. Primary and secondary destabilizing N-terminal residues are denoted by the single-letter abbreviations for amino acids. The shaded ovals indicate the rest of a protein substrate. Conjugation of the primary destabilizing residues Leu or Phe to the secondary destabilizing residues Arg and Lys is catalyzed by L/F–transferase. The degradation of a protein substrate bearing one of the primary destabilizing N-terminal residues (Phe, Leu, Trp, or Tyr) is mediated by the ATP-dependent protease Clp. A question mark indicates a hypothetical recognition step, invoked by analogy to the eukaryotic N-end rule pathway, where a substrate bearing a primary destabilizing N-terminal residue is first bound by a specific recognition protein (*8*).

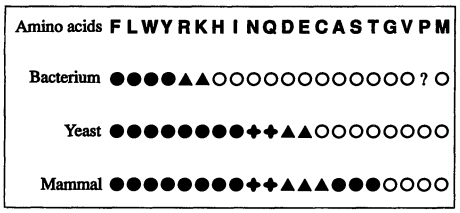

Fig. 4. Comparison of eukaryotic and bacterial N-end rules. Open circles denote stabilizing residues. Filled circles, triangles, and crosses denote, respectively, primary, secondary, and tertiary destabilizing residues in the N-end rules of bacterium (*E. coli*) (Fig. 3), yeast (*S. cerevisiae*) (*2, 4–8*), and mammal (rabbit) reticulocytes (*4–6*). A question mark at Pro indicates its unknown status in the *E. coli* N-end rule (Table 1). Ile is a weakly destabilizing residue in reticulocytes (*6*).

the conjugation of Leu or Phe) to N-terminal Arg or Lys (*13*). However, no significant differences between the degradation kinetics of Arg-βgal and Leu-βgal were observed in wt cells (Fig. 2) (*16*), indicating that the in vivo conjugation of Met, a stabilizing residue in the N-end rule (Table 1), to N-terminal Arg is negligible in comparison to the conjugation of Leu or Phe. [Once conjugated to N-terminal Arg or Lys, the now N-terminal Met residue would not be expected to be removed by the known Met aminopeptidase in *E. coli* (*17*)]. Furthermore, His-βgal was found to be as long-lived as any of the other long-lived X-βgals in *E. coli* (Table 1) (*16*), indicating that the reported ability of N-terminal His to serve as a weak acceptor in reactions catalyzed by L/F–transferase in vitro (*13*) is negligible in vivo.

Given the adenosine triphosphate (ATP) dependence of the N-end rule pathway in eukaryotes (*6*), we determined whether Lon or Clp, the known ATP-dependent proteases in *E. coli* (*1, 18, 19*), are required for the degradation of N-end rule substrates in this organism. We found that all of the normally short-lived X-βgals were metabolically stable in a null *clpA*$^-$ mutant (Figs. 1A and 2) (*16*) that lacks one of the two subunits of Clp (also known as Ti): the ~750-kD, ATP-dependent protease of previously unknown function (*18, 19*). In contrast to the *clpA*$^-$ mutant, mutants in the *lon, hflA, hflB*, or *dnaK* genes of *E. coli*, which encode components of proteolytic and chaperone systems (*1*), were found to be unimpaired in the degradation of N-end rule substrates (*16*).

Thus, the N-end rule operates in both eukaryotes and bacteria. N-terminal Arg and Lys, which are primary destabilizing residues in eukaryotic versions of the N-end rule, are secondary destabilizing residues in *E. coli* (Figs. 3 and 4). This difference between the bacterial and eukaryotic N-end rule pathways is consistent with the observed distribution of the Arg-tRNA–protein transferase, which is confined to eukaryotes, and of the L/F–transferase (Fig. 3), which is present at least in Gram-negative bacteria but is absent from eukaryotes (*13*). The six destabilizing residues of the *E. coli* N-end rule form a subset of the analogous 12-residue set in *S. cerevisiae* (Table 1). The latter is a subset of the 16-residue set of destabilizing residues in the reticulocyte N-end rule (Fig. 4).

One difference between the bacterial and eukaryotic N-degrons is that the *E. coli* N-end rule pathway lacks the ubiquitination step, which is essential in eukaryotes. Whereas the degradation of a short-lived X-βgal in eukaryotes is preceded by its multiubiquitination (*2, 6–8*), no analogous (higher molecular mass) βgal species have been detected by electrophoretic analyses of short-lived X-βgals in *E. coli* (*16*). Moreover, the mutational conversion of the Lys^{15} and Lys^{17} residues in a short-lived X-βgal into Arg residues (which cannot be ubiquitinated) does not impair its N-end rule–mediated degradation in *E. coli* (*16*), whereas the same modification, which eliminates multiubiquitination sites in X-βgal, inactivates its eukaryotic N-degron (*5, 7*). Thus, an N-end rule substrate appears not to undergo a Ub-like modification before its degradation in *E. coli*. One implication of these results is that the bacterial N-degron may be a one-determinant signal, composed of just one (exposed) destabilizing N-terminal residue. Dissection of the manner in which the Ub-dependent steps are bypassed in bacteria should help identify aspects of the Ub function that have kept the Ub system nearly unchanged throughout the evolution of eukaryotes but were apparently dispensable in the course of bacterial evolution.

Our finding that Clp is an essential component of the N-end rule pathway in *E. coli* places Clp within a larger, defined proteolytic system but does not identify physiologically relevant substrates of Clp. Null *clpA*$^-$ mutants appear phenotypically normal and do not stabilize several of the known short-lived proteins in *E. coli* (*18, 19*). Yeast mutants that lack the N-end rule pathway are also viable and nearly normal phenotypically (*8*). With one exception, no physiological substrates of the N-end rule pathway have been identified thus far. The exception is RNA polymerase of the Sindbis virus [a plus-stranded RNA virus (*20*)], which bears an N-terminal Tyr, a destabilizing residue (Fig. 4), and is degraded by the N-end rule pathway (*21*). RNA polymerase is produced by an endoproteolytic cleavage of a viral precursor polyprotein and has been shown to be a short-lived protein in Sindbis virus–infected mammalian cells (*20*). Further identification of physiological substrates of the N-end rule pathway should help clarify its functions.

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11. Ub-X-βgal fusion proteins were expressed in *E. coli* from the pUB23 family of *S. cerevisiae–E. coli*

vectors (*2, 5*) that encode Ub–X-βgals downstream from a galactose-inducible (*GAL10*) yeast promoter. This promoter functions as a weak constitutive promoter in *E. coli* (*10*). The yeast Ub-specific protease Ubp1 was expressed in *E. coli* from the plasmid pJT184, whose P15A replicon is compatible with that of pUB23 (*2, 10*).

12. *Escherichia coli* BW13711 [$F^-\lambda^-$K12 $\Delta(lac)_{X74}$] [W. W. Metcalf, P. M. Steed, B. L. Wanner, *J. Bacteriol.* **172**, 3191 (1990)] carrying pUB23-X, which expressed Ub–X-βgal, and pJT184, which expressed Ubp1 (*11*), were grown at 36°C to an optical density at 600 nm of ~0.3 in M9 medium supplemented as described (*10*). Cells in 5 ml of the culture were collected by centrifugation, resuspended in M9 medium (0.25 ml), and incubated at 36°C for 10 min. [^{35}S]Translabel (ICN Biomedicals) (0.1 mCi) was then added, and cells were incubated for 2 min. Chase solution (12.5 μl) containing L-Met (40 mg/ml) and L-Cys (10 mg/ml) was added, and a portion of the cell suspension (50 μl) was withdrawn, transferred into a chilled tube containing lysis buffer (50 μl) [4% SDS and 125 mM tris-HCl, (pH 6.8)], and frozen by immersion in liquid N_2. The rest of the cell suspension was incubated at 36°C with intermittent mixing; aliquots (50 μl) were withdrawn at various times after the pulse and processed as above. The chase points in Fig. 1A were 3, 10, 30, and 60 min for long-lived X-βgals and 3, 5, 10, and 30 min for short-lived X-βgals. Conditions of the pulse-chase assay did not perturb the N-end rule pathway because cells incubated under these conditions for 1 hour before the pulse labeling did not alter kinetics of X-βgal degradation. The frozen samples were heated at 100°C for 4 min. Each sample was then added to immunoprecipitation (IP) buffer (1 ml) [1% Triton X-100, 0.5% deoxycholate, 0.15 M NaCl, 5 mM EDTA, 50 mM Hepes, (pH 7.5), plus freshly added 1 mM phenylmethylsulfonyl fluoride] and centrifuged at 12,000*g* for 10 min at 4°C. The supernatant (0.9 ml) was added to a solution (1 μl) containing a monoclonal antibody to βgal (2 mg/ml; Promega) and incubated at 0°C for 1 hour. A protein A–agarose suspension (Repligen) (10 μl) was then added; the suspension was incubated with rocking for 30 min at 4°C and centrifuged for 15 s at 12,000*g*. The pellet was washed three times with IP buffer containing 0.1% SDS, dissolved in SDS-containing sample buffer, and subjected to electrophoresis in a SDS–6% polyacrylamide gel (*10*). The gels were washed in 25% CH_3OH and 10% CH_3COOH for 20 min, dried, autoradiographed, and quantified (*22*).
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15. We produced *E. coli* strains TS353 (MC1061 *aat3::mini-tet*) and TS354 (MC1061 *clpA14::mini-tet*) by transposon mutagenesis, using a *mini-tet* derivative of the transposon Tn*10* [J. C. Way, M. A. Davis, D. Morisato, D. E. Roberts, N. Kleckner, *Gene* **32**, 369 (1984)]. A X-Gal plate test (*8*) was used to isolate transposon insertions that stabilized Arg-βgal. We cured the mutants thus obtained of pUB23-R [the plasmid expressing Ub–Arg-βgal (*11*)] and retransformed the mutants with pUB23-L (the plasmid expressing Ub–Leu-βgal) to distinguish between mutants in the *aat* and *clpA* genes. Details of the screen, gene mapping, *mini-tet* insertion sites, and the nucleotide sequence of the cloned *aat* gene will be published elsewhere.
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22. After electrophoresis of ^{35}S-labeled proteins, the gels were dried and exposed for ~24 hours to freshly erased storage phosphor screens. We used a PhosphorImager (Molecular Dynamics) to quantify autoradiographic images stored by the screens, as described [R. F. Johnson, S. C. Picket, D. L. Barker, *Electrophoresis* **11**, 355 (1990)].
23. We thank B. Wanner for the BW13711 strain; D. Parcell, R. Sauer, W. Walter, C. Gross, and B. Bachman for other *E. coli* strains; G. Walker and members of his laboratory for advice; J. Essigman for making PhosphorImager available to us; and B. Doran for secretarial assistance. Supported by NIH grants DK39520 and GM31530 (to A.V.). T.E.S. is a Merck Fellow of the Helen Hay Whitney Foundation.

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A Neural Mechanism for Working and Recognition Memory in Inferior Temporal Cortex

EARL K. MILLER, LIN LI,* ROBERT DESIMONE†

Inferior temporal (IT) cortex is critical for visual memory, but it is not known how IT neurons retain memories while new information is streaming into the visual system. Single neurons were therefore recorded from IT cortex of two rhesus monkeys performing tasks that required them to hold items in memory while concurrently viewing other items. The neuronal response to an incoming visual stimulus was attenuated if it matched a stimulus actively held in working memory, even when several other stimuli intervened. The neuronal response to novel stimuli declined as the stimuli became familiar to the animal. IT neurons appear to function as adaptive mnemonic "filters" that preferentially pass information about new, unexpected, or not recently seen stimuli.

THE IT CORTEX OF BOTH HUMAN and nonhuman primates is critical for visual memory. In monkeys, removal of IT cortex impairs performance of tasks that require judgments of either stimulus recency (working memory) or stimulus familiarity (recognition memory). In the absence of IT cortex, the memory of a visual stimulus decays significantly over the course of a minute or two and may decay even more rapidly if the subject views other stimuli during the retention interval (*1*). It is not understood how memories for stimuli are retained in IT cortex, particularly as new stimuli continuously enter the visual system and, presumably, activate the same IT neurons involved in the retention of memories.

We therefore recorded from neurons in the anterior IT cortex of two rhesus monkeys that were required to retain items in memory while viewing a series of other stimuli (*2*). We used two variants of a delayed matching-to-sample task designed to explore the role of IT neurons in either working or recognition memory. On each trial, while the monkey held a bar and maintained fixation on a fixation target (*3*), a sample stimulus was presented at the center of gaze followed by sequential presentation of one to five test stimuli. All sample and test stimuli were on for 500 ms each, separated by 700-ms delays. The monkey held onto the bar until one of the test stimuli in the sequence matched the sample, at which point it released the bar for an orange juice reward, terminating the trial (*4*). The nonmatching stimuli that intervened between the sample and final matching stimulus differed from each other and from the sample. The stimuli were color-digitized, common objects presented on a computer graphics display.

In the working memory procedure, we asked whether the particular sample stimulus the animal held in memory on each trial affected how cells processed new inputs (the test stimuli). Each cell was studied with six familiar stimuli, each of which appeared as the sample and matching stimulus on some trials, and as a nonmatching stimulus on others, for a total of about 360 trials per cell (*5*).

We initially recorded from 146 IT neurons and found that nearly half responded selectively not only to particular test items but also according to whether the test items matched the sample item held in memory. Two-way analyses of variance (ANOVAs) were applied to the responses of each cell separately, with the six test items and the matching-nonmatching status as factors. The responses to matching stimuli that appeared after four intervening items were excluded from all statistical analyses, so that the mean number of intervening items preceding matching and nonmatching stimuli

Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

*Present address: Department of Psychology, University of California, Los Angeles, CA 90024.
†To whom correspondence should be addressed.