TABLE I

The N-end rule in yeast and in mammalian reticulocytes

The *in vivo* half-lives of *X*-βgal test proteins in the yeast *S. cerevisiae* were determined as previously described (Bachmair *et al.*, 1986). The yeast N-end rule as reported by Bachmair *et al.* (1986) is updated here by inclusion of the four remaining amino acids, Cys, His, Trp, and Asn (see also Bachmair and Varshavsky, 1989). The half-lives of purified, ^{35}S-labeled *X*-βgal test proteins in the ATP-supplemented reticulocyte extract were estimated from semilogarithmic plots of the degradation time courses in Fig. 4 (see main text). The half-lives thus determined were reproducible among different preparations of the extract and of *X*-βgal test proteins (data not shown). Amino acid sequencing of reisolated *X*-βgal proteins is described under "Experimental Procedures." When a mixture of two sequences was obtained, both of the deduced sequences (separated by +) were included into the table.

Residue *X* in Ub-*X*-βgal	Half-life of *X*-βgal		Amino terminus of reisolated *X*-βgal as determined by protein sequencing	
	Yeast (*S. cerevisiae*) *in vivo*	Mammalian reticulocytes *in vitro*	Yeast *in vivo*	Reticulocytes *in vitro*
Val	>20 h	100 h		Val-βgal[b,c]
Met	>20 h	30 h	Met-βgal[a]	Met-βgal[b,c]
Gly	>20 h	30 h		Gly-βgal[b,c]
Pro	>20 h[d]	>20 h[d]		Pro-βgal[d]
Ala	>20 h	4.4 h	Ala-βgal[e]	Ala-βgal[b,f]
Ser	>20 h	1.9 h	—[g]	Ser-βgal[b,f]
Thr	>20 h	7.2 h	Thr-βgal[e]	Thr-βgal[b,f]
Cys	>20 h	1.2 h		[?]-βgal[e,h]
Ile	30 min	20 h	Ile-βgal[e,i]	Ile-βgal[b,c]
Glu	30 min	1.0 h	Arg-Glu-βgal[i]	Glu-βgal + Arg-Glu-βgal[b] Arg-Glu-βgal[f]
His	10 min	3.5 h		His-βgal[b]
Tyr	10 min	2.8 h	Tyr-βgal[e,i]	Tyr-βgal[b]
Gln	10 min	0.8 h	[?]-Glu-βgal[j]	[?]-Glu-β-gal + Glu-βgal[b,k] Arg-Glu-βgal[f]
Asp	3 min	1.1 h	Arg-Asp-βgal[i]	Asp-βgal + Arg-Asp-βgal[b] Arg-Asp-βgal[f]
Asn	3 min	1.4 h	Arg-Asp-βgal[i]	Asn-βgal + Asp-βgal[b] Asn-βgal + Arg-Asp-βgal[f]
Phe	3 min	1.1 h		Phe-βgal[b]
Leu	3 min	5.5 h		Leu-βgal[b]
Trp	3 min	2.8 h		Trp-βgal[b]
Lys	3 min	1.3 h		Lys-βgal[b]
Arg	2 min	1.0 h		Arg-βgal[b]

[a] Determined by radiochemical sequencing (Bachmair *et al.*, 1986).

[b] This *X*-βgal protein was incubated in ATP-depleted reticulocyte extract for 20 min at 37°C before reisolation and sequencing.

[c] This *X*-βgal test protein was incubated in ATP-supplemented reticulocyte extract for 1 h at 37°C before reisolation and sequencing.

[d] In both yeast cells and reticulocyte extract, Ub-Pro-βgal is deubiquitinated approximately 20 times more slowly than are the rest of the Ub-*X*-βgal fusion proteins (see main text). Pro-βgal, the product of slow deuibiquitination of Ub-Pro-βgal, is a long-lived protein in both yeast cells and reticulocyte extract.

[e] The *S. cerevisiae* strain used for expression of this *X*-βgal protein was BWG-9a-1 (*MATα, his4, ade6, ura3*).

[f] This βgal protein was incubated for 2 h at 37°C in ATP-supplemented reticulocyte Fraction II before reisolation and sequencing.

[g] No signal was seen upon sequencing of Ser-βgal reisolated from yeast, strongly suggesting that the protein's amino terminus was blocked. Note that Ser-βgal was not blocked when reisolated from ATP-supplemented reticulocyte extract.

[h] Cys-βgal was incubated in ATP-depleted reticulocyte extract for 30 min at 37°C before reisolation and sequencing. The amino-terminal Cys, unmodified by alkylation before sequencing, could not be identified by the chromatographic procedures used; however, the second and subsequent sequencing steps unambiguously identified the protein as βgal.

[i] The *S. cerevisiae* strain used for expression of this *X*-βgal protein was a mutant (obtained in the background of the BWG-9a-1 strain) in which all of the otherwise short-lived (deubiquitinated) *X*-βgal test proteins are metabolically stable, whereas Ub-Pro-βgal is still short-lived (I. Wünning, A. Bachmair, and A. Varshavsky, unpublished data). This mutant (whose use allowed the isolation of the otherwise short-lived *X*-βgal proteins in quantities sufficient for sequencing) retains both the intact "downstream" degradation pathway and the Ub-*X*-βgal deubiquitinating activity but is impaired in the amino-terminal recognition of at least the *X*-βgal proteins.

[j] The amino-terminal residue of this sequence could not be identified unambiguously with the amount of βgal used (~15 pmol), but, from the data obtained, was most likely Arg. The data clearly identified Glu as the second residue.

[k] The frame-shifted sequence (?)-Glu-βgal was the more abundant (~90%) of the two sequences present. With the amount of βgal used (~15 pmol), the amino-terminal residue of this sequence could not be identified unambiguously but, from the data obtained, was most likely Arg.