## Supplementary Figures and Tables

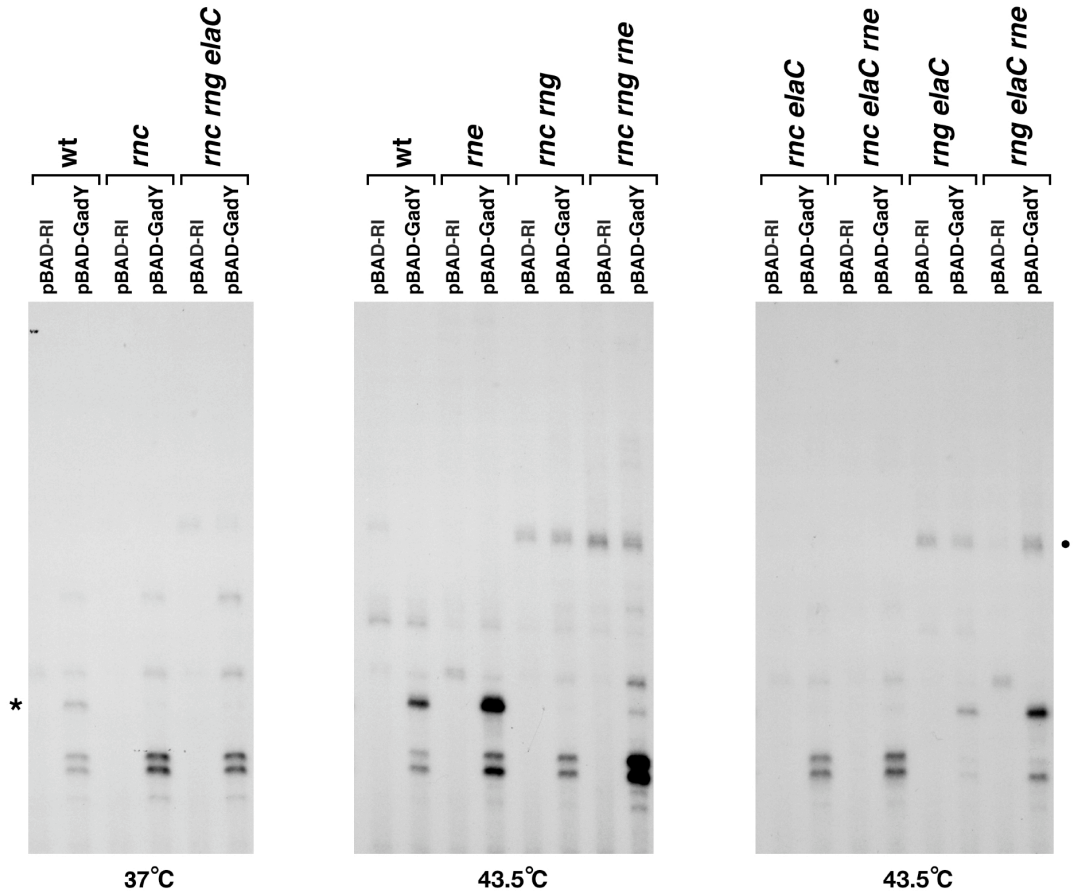


**Fig. S1.** GadY-directed processing in RNase triple mutant strains. Total RNA was isolated from wild type and RNase mutant derivatives of the $lacZ_{gadX}$ reporter strain harboring pBAD-RI and pBAD-GadY, 20 min following the addition of arabinose to 0.2% to actively growing cultures ($OD_{600} \approx 0.7$). For the strains carrying the temperature sensitive *rne* alleles, the culture was shifted to the non-permissive temperature (43.5°C) for 30 min prior to induction. Samples were analyzed as in Fig. 4. The band decreased in *rnc* mutant strains is indicated by the asterick, and the extra band present in *rng* mutant strains is indicated by the bullet.

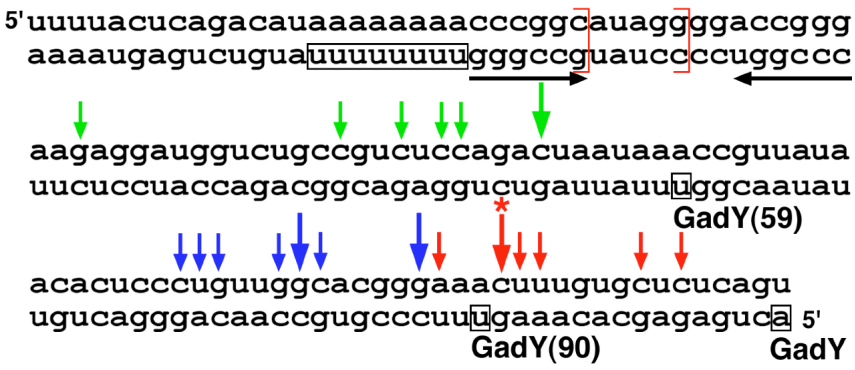


**Fig. S2.** 5'-ends detected by primer extension and 3'-ends detected by 3' RACE for products of in vitro assay with partially purified RNase III. 5' ends are indicated by arrows, 3' ends are indicated by brackets, and end specific to the wild type strain is indicated by the asterick. Red denotes the GadY-dependent ends, blue denotes the GadY(90)-dependent ends and green denotes the pRI-GadY(59)-dependent ends.

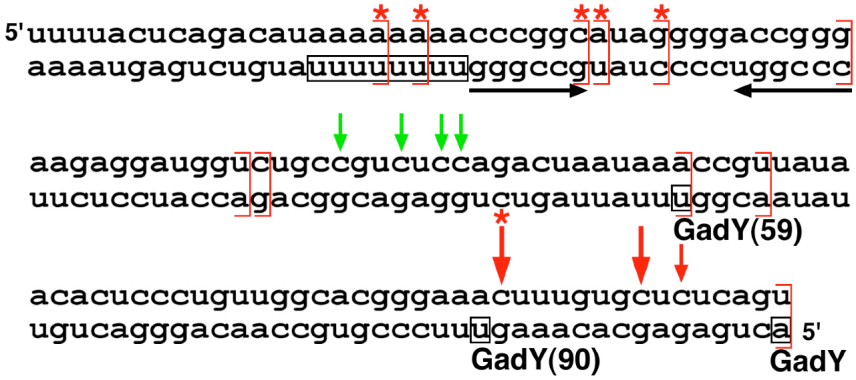


**Fig. S3.** 5'-ends detected by primer extension assays and 3'-ends detected by 3' RACE assays for products of in vivo cleavage in wild type and *rnc* mutant strains. 5' ends are indicated by arrow, 3' ends are indicated by brackets, and ends specific to the wild type strain are indicated by astericks. Red denotes the GadY-dependent ends and green denotes the pRI-GadY(59)-dependent ends.

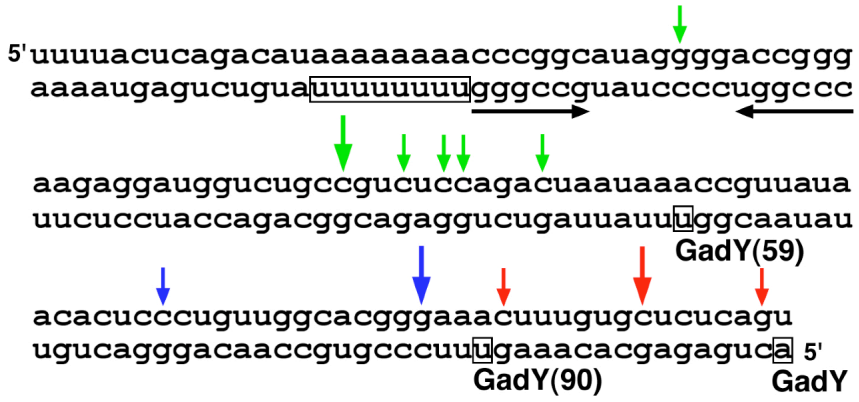


**Fig. S4.** 5'-ends detected by primer extension assays for products from in vitro assay with *rnc* mutant extracts. 5' ends are indicated by arrows. Red denotes the GadY-dependent ends, blue denotes the GadY(90)-dependent ends and green denotes the pRI-GadY(59)-dependent ends.

**Table S1.** Oligonucleotides used in study

| Name | Sequence (5'-3', restriction sites underlined) | Use |
|---|---|---|
| GadY-S1 | cacacagaattctttattagtctggagacggcagac | pRI-GadY(59), pBAD-GadY(59) PCR |
| GadY-A1 | cacacaaagcttaaaaaaaaacccggcatagggggaccg | pRI-GadY(59) |
| lacZ-S3 | ccacaggatccggccgattcattaatgcagc | pACYC-*lacZ* PCR |
| lacZ-A3 | cacacaaagctttattttttgacaccagacca | pACYC-*lacZ* PCR |
| gadW-A1 | gcagcagaatactctcgttatgcagcg | pACYC-$lacZ_{gadX}$ PCR, *gadW* Northern detection |
| gadX-S1 | ctagcccgaatcgccagcga | pACYC-$lacZ_{gadX}$ PCR |
| lacZ-S10 | cacaagatcttcccagtcacgacgttgtaa | pACYC-$lacZ_{gadX}$ PCR |
| lacZ-A6 | cacactcgagaaccctggcgttacccaactt | pACYC-$lacZ_{gadX}$ PCR |
| gadY-QC1 | ggaagaggatggtctgccgtc | pACYC-$lacZ_{gadX}$ PCR |
| gadY-QC2 | gacggcagaccatcctcttcc | pACYC-$lacZ_{gadX}$ PCR |
| IG-S1 | cacaagatcttttactcagacataaaaaa | pACYC-$lacZ_{gadX}$ PCR |
| IG-A2 | cacactcgagactgagagcacaaagtttcc | pACYC-$lacZ_{gadX}$ PCR |
| AZ784 | cgcaactctctactgtttgaattcacccgtttttttgggcta | pBAD-RI PCR |
| AZ785 | tagcccaaaaaaacgggtgaattcaaacagtagagagttgcg | pBAD-RI PCR |
| JK116 | gttatattgaattcactgagagcac | pBAD-GadY PCR |
| JK117 | cttactgagagcgaattctttcccgtgc | pBAD-GadY(90) PCR |
| JK118 | gaataagattataagctttactcagac | pBAD-GadY, pBAD-GadY(90), pBAD-GadY(59) PCR |
| cat-S2 | gtcatcctgcccgtagaacatcacacattatcatcctgttctcccgcttcggaagatcacttcgcagaa | GSO129 PCR |
| Px-cat-S1 | ttaataaacagtaatatgtttatgtaatattaagtcaactatggagaaaaaaatcactgg atatac | GSO129 PCR |
| gfp-S3 | gccgggaaatacccagcctc | GSO403 PCR |
| gfp-A2 | tggcagttccctactctcgcaggtcagctaattaagcttat | GSO403 PCR |
| gfp-S2 | taacactccctgttggcacgggaaactttgtgctctcagtggatccgtcgactctagagg | GSO403 PCR |
| PS2-X-A1 | atgtttataaaaaaatggctgatcttatttccagtaaaagcatatgaatatcctccttagt | GSO403, GSO404 PCR |
| pKD4-S2 | tgcgagagtagggaactgcca | GSO403, GSO404 PCR |
| pKD4-A2 | gttgtggacaacaagccagg | GSO403, GSO404 PCR |
| lacZ-234-R | tggcagttccctactctcgcatgtttgacagcttatcatcg | GSO404 PCR |
| lacZ-S3 | cacaggatccggccgattcattaatgcagc | GSO404 PCR |
| GadX-LacUP | tcctgcccgtagaacatcacacattatcatcctgttctcccgcttccgcaattaatgtgagttagctcac | GSO404 PCR |
| PS1-rng | atgacggctgaattgttagtaaacgtaacgccttcggtgtaggctggagctgcttc | GSO410 PCR (Δ*rng*) |
| PS2-rng | gcccagcatctgtttacatcattacgacgtcaaactgctcctgcatatgaatatcctccttagt | GSO410 PCR (Δ*rng*) |
| PS1-rnlA | attgtagagtttccccatatgtttctatgggatccaggaagtgtaggctggagctgcttc | GSO411 PCR (Δ*rnlA*) |
| PS2-rnlA | gacagcttttagagcccctgagacgttaattccggtgattcatatgaatatcctccttagt | GSO411 PCR (Δ*rnlA*) |
| mh686 | tatgccttatactagccggaatatttttttgaaacgtgatgaactcgtgtaggctggagctgcttc | GSO412 PCR (Δ*elaC*) |
| mh687 | ataacagggcaagtttcgccctgtttttaataataagcagagttaatgaatatcctccttag | GSO412 PCR (Δ*elaC*) |
| gadX-T7 | cttgaattctaatacgactcactatagggagagattgtcgaaccgtgac | *gadX* in vitro transcription template PCR |
| gadX-T7-R | gcacgaatatattttcgcac | *gadX* in vitro transcription template PCR |
| gadY-T7 | cttgaattctaatacgactcactatagggactgagagcacaaagtttcc | GadY in vitro transcription template PCR |
| gadY90-T7 | cttgaattctaatacgactcactatagggtttcccgtgccaacagggag | GadY(90) in vitro transcription template PCR |
| gadY59-T7 | cttgaattctaatacgactcactatagggtttattagtctggagacggc | GadY(59) in vitro transcription template PCR |
| gadY-T7-R | aaaaaaaacccggcatagggggaccg | GadY, GadY(90), GadY(59) in vitro transcription template PCR |
| gfp-R | tgttggccatggaacaggtagttttccagt | *gfp* Northern detection |
| gadX-A1 | gcttgcatccgcaaaaaccaggtcaccgcc | *gadX* Northern detection |
| cat-A1 | aggccgtaatatccagctgaacggtctggt | *cat* Northern detection |
| lacZ-R2 | gggcctcttcgctattacgccagctggcga | *lacZ* primer extension |
| GadW-A2 | acgaatatattttcgcacagcgtatagctt | *gadW* primer extension |
| EF584 | gggtttattgagaatatgtttttcgtctcagcc | *cat* 3' RACE |
| EF607 | cttggacacaaattggaatacaactataactc | *gfp* 3' RACE |

**Table S2.** Plasmids used in study

| Name | Resistance markers | Source or reference |
|---|---|---|
| pRI | ($Amp^R$) | [1] |
| pRI-GadY | ($Amp^R$) | [1] |
| pRI-GadY(90) | ($Amp^R$) | This study |
| pRI-GadY(59) | ($Amp^R$) | This study |
| pBAD18 | ($Amp^R$) | [2] |
| pBAD-RI | pBAD18 with an EcoRI site at the transcription start site ($Amp^R$) | This study |
| pBAD-GadY | ($Amp^R$) | This study |
| pBAD-GadY(90) | ($Amp^R$) | This study |
| pBAD-GadY(59) | ($Amp^R$) | This study |
| pACYC184 | ($Cm^R$, $Tet^R$) | [3] |
| pACYC-*lacZ* | ($Cm^R$) | This study |
| pACYC-$lacZ_{gadX}$ | ($Cm^R$) | This study |
| p$lacZ$-$gadX_{gadY\text{-}10\ \text{mutant}}$ | ($Cm^R$) | [1] |
| pKD3 | ($Amp^R$,$Cm^R$) | [4] |
| pKD4 | ($Amp^R$, $Kan^R$) | [4] |
| pCP20 | ($Amp^R$) | [5] |
| pMG47 | ($Amp^R$, $Kan^R$) | [6] |

**Table S3.** Strains used in study

| Name | Relevant genotype | Source or reference |
|---|---|---|
| MG1655 | F- lambda- ilvG- rfb-50 rph-1 | lab stock |
| GSO109 | MG1655 *gadY-10* | [1] |
| NM500 | MG1655 mini-λ-*tet* | N. Majdalani |
| NM700 | MG1655 Δ*lacZ*nm3, mini-λ-cm | N. Majdalani |
| AC22 | *zce726::*Tn*10 rne3071* | [7] |
| NHY322 | zci-501::Tn10 (tet) rnpA49 | [8] |
| EK442 | Δ*gadXW::kan* | [9] |
| NB478 | *rnc::cat* | [10] |
| HT115 | Δ*rnc::Tn10* (tet) | [11] |
| MIC3009 | *rnhA*::*cat* | [12] |
| GSO129 | MG1655 Δ*gadX*:: $P_{cat}$-*cat, gadY-10* | This study |
| GSO403 | MG1655 Δ*gadX*:: $P_{cat}$-*cat* Δ*gadW*::*gfp-rrnB* T1 T2 | This study |
| GSO404 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ | This study |
| GSO405 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* | This study |
| GSO406 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ *zce726*::Tn*10* | This study |
| GSO407 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ *zce726*::Tn*10 rne3071* | This study |
| GSO408 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ *zci-501::Tn10* | This study |
| GSO409 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ *zci-501::Tn10 rnpA49* | This study |
| GSO410 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rng::kan* | This study |
| GSO411 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnlA::kan* | This study |
| GSO412 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*elaC::kan* | This study |
| GSO413 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat zce726*::Tn*10* | This study |
| GSO414 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat zce726*::Tn*10 rne3071* | This study |
| GSO415 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rng::kan zce726*::Tn*10* | This study |
| GSO416 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rng::kan zce726*::Tn*10 rne3071* | This study |
| GSO417 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat zci-501*::Tn*10* | This study |
| GSO418 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat zci-501*::Tn*10 rnpA49* | This study |
| GSO419 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*rng::kan* | This study |
| GSO420 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*rnlA::kan* | This study |
| GSO421 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc*::Tn*10* | This study |
| GSO422 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc*::Tn*10* Δ*rnhA::cat* | This study |
| GSO423 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*rng::kan zce726*::Tn*10* | This study |
| GSO424 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*rng::kan zce726*::Tn*10 rne3071* | This study |
| GSO425 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rng::kan* Δ*elaC::cat zce726*::Tn*10* | This study |
| GSO426 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rng::kan* Δ*elaC::cat zce726*::Tn*10 rne3071* | This study |
| GSO427 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*elaC::kan zce726*::Tn*10* | This study |
| GSO428 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*rnc::cat* Δ*elaC::kan zce726*::Tn*10 rne3071* | This study |
| GSO429 | MG1655 Δ*lacZ* Δ*gadXY*::$P_{lac}$-$lacZ_{gadX}$ Δ*elaC* Δ*rnc::cat* Δ*rng::kan* | This study |
| GSO430 | MG1655 Δ*gadXW::kan* | This study |
| GSO431 | MG1655 Δ*gadXW::kan* Δ*rnc::ca*t | This study |
| GSO432 | MG1655 Δ*gadX*:: $P_{cat}$-*cat* Δ*gadW::gfp-rrnB* T1 T2 *rnc::Tn10-tet* | This study |

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