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4 Title: AS202

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6 Highlights:

7 Depex2 proximity labeling can be applied to RN Examples in the internet in the internet of the 48202

2 Corresponding Author: Schrader@wayne.edu

4 Author: Schrader@wayne.edu

4 Highlights:

7 Dentex Proximity labeling can be applied to

8 Dentex Proximity labeling ca 3 departments of Chemistry and Biological Sciences and Biological Sciences and Highlights:

3 Dept APEX2 proximity labeling can be applied to RNA in bacteria

3 Dept APEX2 workflow requires less material and time than curr 48202 4 Example Muslim Complete Maynesian

5 APEX2 proximity labeling can be applied

8 D APEX2 RNA labeling reactions occur on t

9 D APEX2 workflow requires less material a

0 D Alkyne-Phenol APEX2 substrate provides

1 Motivati 6 Highlights:

7 D APEX2

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1 Motivation:

2 Studie:

to subcellular

4 bacteria is cul

5 isolate ribonu

6 major challen

7 typically last f

8 localization or studying RNA 7 APEX2 proximity labeling can be applied to RNA in bacteria 9
■ APEX2 workflow requires less material and time than current method

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■ Alkyne-Phenol APEX2 substrate provides flexibility with click-chem

Motivation:

Studies over the past several years have shown that distinct 9 A Alternative and APEX2 substrate provides flexibility with click-chemistry

9 A Altyne-Phenol APEX2 substrate provides flexibility with click-chemistry

9 Motivation:

9 Studies over the past several years have shown th 11 Motivation:

12 Studies over the past several years have shown that distinct RNAs can b

13 to subcellular locations in bacterial cells. The ability to investigate localized RN

14 bacteria is currently limited to imagi 11 Frottvation:
12 Studies
13 to subcellular
15 isolate ribonuc
16 major challeng
17 typically last fo
18 localization or
19 studying RNA l
20 Summary:
21 Rapid s
22 bacterial cells.
12 in eukaryotic c
24 different subce
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13 to subcellular locations in bacterial cells. The ability to investigate localized RNAs in

14 bacteria is currently limited to imaging-based approaches or to laborious procedures to

15 isolate ribonucleoprotein complex bacteria is currently limited to imaging-based approaches or to laborious procedures
isolate ribonucleoprotein complexes by grad-seq. HITS-CLIP, or Rloc-seq. However,
major challenge in studying mRNA localization in bacter 15 isolate ribonucleoprotein complexes by grad-seq, HITS-CLIP, or Rloc-seq. However, a
16 major challenge in studying mRNA localization in bacterial cells is that bacterial mRNAs
17 typically last for only a few minutes in major challenge in studying mRNA localization in bacterial cells is that bacterial mRNA
typically last for only a few minutes in the cell, while experiments to investigate their
localization or interaction partners can tak 17 typically last for only a few minutes in the cell, while experiments to investigate their
18 localization or interaction partners can take much longer. Therefore, rapid methods of
19 studying RNA localization are needed 18 tocalization or interaction partners can take much longer. Therefore, rapid methods
19 typical studying RNA localization are needed to bridge this technical challenge.
17 Rapid spatially controlled methods are needed to studying RNA localization are needed to bridge this technical challenge.

20 Summary:

21 Rapid spatially controlled methods are needed to investigate RNA localization in

22 bacterial cells. APEX2 proximity labeling was s 19 Summary:

19 Summary:

21 Rapid spatially controlled methods are needed to investigate RNA

22 bacterial cells. APEX2 proximity labeling was shown to be adaptable to r

19 in eukaryotic cells, and through the fusion of 20 Summary:

21 Rapid:

22 bacterial.cell:

23 in eukaryotic

24 different subc

26 APEX2 fusion

27 formation.AP

28 sub-minute ti

29 bacteria.Alk_!

30 copper cataly

29 pacteria.Alk!

29 copper cataly 22 bacterial cells. APEX2 proximity labeling was shown to be adaptable to rapid RNA labeli

23 in eukaryotic cells, and through the fusion of APEX2 to different proteins targeted to

24 different subcellular locations, has 22 in eukaryotic cells, and through the fusion of APEX2 to different proteins targeted to
24 different subcellular locations, has been useful to identify RNA localization in these cells.
25 Therefore, we adapted APEX2 prox 24 different subcellular locations, has been useful to identify RNA localization in these
25 Therefore, we adapted APEX2 proximity labeling of RNA to bacterial cells by generati
26 APEX2 fusion to the RNase E gene, which i Therefore, we adapted APEX2 proximity labeling of RNA to bacterial cells by generating an
26 APEX2 fusion to the RNase E gene, which is necessary and sufficient for BR-body
27 formation. APEX2 fusion is minimally perturbat 26 APEX2 fusion to the RNase E gene, which is necessary and sufficient for BR-body
27 formation. APEX2 fusion is minimally perturbative and RNA can be rapidly labeled on the
28 sub-minute timescale with Alkyne-Phenol, outp 27 Formation. APEX2 fusion is minimally perturbative and RNA can be rapidly labeled
28 sub-minute timescale with Alkyne-Phenol, outpacing the rapid speed of mRNA de
29 bacteria. Alkyne-Phenol provides flexibility in the ov 28 sub-minute timescale with Alkyne-Phenol, outpacing the rapid speed of mRNA decay in
29 bacteria. Alkyne-Phenol provides flexibility in the overall downstream application with
20 copper catalyzed click-chemistry for down 28 sub-minute and Controllect minimally the rather typescale with all downstream application with
20 societia. Alkyne-Phenol provides flexibility in the overall downstream application with
20 sopper catalyzed click-chemist 29 backet alkyne-Phenol provides flexibility in the overall alternative provides fluorescent dy-
29 copper catalyzed click-chemistry for downstream applications, such as fluorescent dy-
20 copper catalyzed click-chemistry

provides a useful method for studying RNA localization in bacteria.

32 provides a useful method for studying RNA localization in bacteria.

33 Introduction:

33 Bobal level, many mRNAs have been found to be associated wit 22 provides a unit metallication in the studies and the studies and the studies and the studies a useful metallic membrane, or cell poles¹⁻⁴ and it has been hypothesized that mRNA important for proper gene expression. Fo 33 Introduction:
34 Bacterial
35 global level, mar
36 membrane, or ce
37 inportant for prc
38 jejuni localizes to
39 addition, biomol
40 assembled throu
41 multiple involved
42 RNAs to biomole
43 GRAD-sec
identifying RNAs
 35 global level, many mRNAs have been found to be associated with the nucleoid,
36 membrane, or cell poles^{t-4} and it has been hypothesized that mRNA localization can be
37 important for proper gene expression. For examp 36 membrane, or cell poles¹⁴ and it has been hypothesized that mRNA localization
37 important for proper gene expression. For example, Flagellin mRNA in *Campylo*
38 addition, biomolecular condensates, which are non-mem

membrane, or cell poles² - and it has been hypothesized that mRNA localization can be

important for proper gene expression. For example, Filagellin mRNA in Campylobacter

addition, biomolecular condensates, which are no structure through the cell poles which may facilitate cotranslational flagellar assembly
addition, biomolecular condensates, which are non-membrane bound organelles ofter
assembled through phase separation, have been rapi *iejuni* localizes to the cell poles which may identiate corranstational riagatiar assembly. In addition, biomolecular condensates, which are non-membrane bound organelles often a may assembled through phase separation, h 39 assembled through phase separation, have been rapidly expanding in bacterial cells, with multiple involved in RNA metabolism^{4,6}. Yet the functional significance of localization of RNAs to biomolecular condensates in

41 multiple involved in RNA metabolism⁴⁸. Yet the functional significance of localization of
42 RNAs to biomolecular condensates in bacteria has just started to be explored.
43 GRAD-seq, HITS-CLIP, and Rloc-seq methods multiple involved in RNA metabolism¹⁶³, vet the functional significance of localization of

RNAs to biomolecular condensates in bacteria has just started to be explored.

GRAD-seq, HITS-CLIP, and Rloc-seq methods^{1,2,8} 43 GRAD-seq, HITS-CLIP, and Rioc-seq methods^{1,7,8} have been successful
dentifying RNAs associated with specific RNPs or localized to certain subcellu
locations. However, a major difficulty is that bacterial mRNAs are ver GRAD-seq, HITS-CLIP, and Rioc-seq methods^{35,66} have been successful at
identifying RNAs associated with specific RNPs or localized to certain subcellular
locations. However, a major difficulty is that bacterial mRNAs are 45 locations. However, a major difficulty is that bacterial mRNAs are very short lived,

46 these methods require labeling or isolation times much longer than a typical mRN

47 life, leading to the potential for false neg 46 these methods require labeling or isolation times much longer than a typical mRNAs hife, leading to the potential for false negatives. Therefore, rapid methods are needed tisolate the associated labile bacterial mRNA be 48 isolate the associated labile bacterial mRNA before they are degraded.

49 APEX2 proximity labeling allows for spatially controlled and rapid RNA labeling^{3,16}

50 making it a useful method to assay for localized bacte 49 APEX2 proximity labeling allows for spatially controlled and rapid making it a useful method to assay for localized bacterial RNAs. In this

show that APEX2 proximity labeling can be adapted to bacterial conden

the c APEX2 proximity labeling allows for spatially controlled and rapid RNA labeling.²⁰¹,

So making it a useful method to assay for localized bacterial RNAs. In this menuscript, we

So the core BR-body scaffold RNase E to AP 51 show that APEX2 proximity labeling can be adapted to bacterial condensates via fusion
52 the core BR-body scaffold RNase E to APEX2. RNA can be labeled robustly by APEX2 with
53 H₂O₂ and Alkyne-Phenol, allowing cop 52 show that APEX2 to different and the set of APEX2. ANA can be tableted robustty by APEX2 with
 H_2O_2 and Alkyne-Phenol, allowing copper catalyzed click chemistry with a variety of azides

such as Cy5-azide. This pro 52 H₃O₂ and Alkyne-Phenol, allowing copper catalyzed click chemistry with a variety of azides

54 H₃O₂ and Alkyne-Phenol, allowing copper catalyzed click chemistry with a variety of azides

54 such as Cy5-azide. T 54 Such as Cy5-azide. This procedure can be used to rapidly label bacteriat RNAs by APEX2

55 on the sub-minute timescale, and which can be rapidly purified via copper catalyzed click

56 chemistry conjugation of biotin-pe

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image
included and the ical density (
was added and
mM H_2O_2 solutions then addentical density
is carried out
 O_2 added (0.5 in added (0.5 in and the cell
, and the cell roximity labe
to quench the 60 62 APEX2, Alkyne-Phenol, and H2C

63 NA1000 and Js767 (RNE-Apex2F

inoculated into 5 mL M2G the ne

65 of 1.2 where BR-bodies were str

incubated for 30 minutes in a sh

67 (996 µL H₂O + 6 µL H₂O₂, 50%) w

45 second 62 APEX2, Alkyne-Phenol, and H2O2 are needed for RNA proximity labeling NA 1000 and Js/b/ (RNE-Apex2FigC-2) were grown in PYE at 28°C overnight and re-

incoulated into 5 mL M2G the next day. Once the cultures reached an optical dens

66 of 1.2 where BR-bodies were strongly induced, 2.5 mM of

alkyne-phenol and H_2O_2 added, different concentrations of alkyne-phenol added (0.5 mM
and 1 mM), and different H_2O_2 exposures (15 seconds, 30 seconds, and 60 seconds). 4 mL

of cell culture were then quickly transferred into two 2 mL Eppendorf tubes and centrifuged

pellets were resuspended in 1 mL of 65°C pre-warmed TRIzol. Because proximity labeling

65 of 1.2 where BR-bodies were strongly induced, 2.5 mM of alkyne-phenol was added and
66 incubated for 30 minutes in a shaker incubator at 28°C at 200 rpm. A 100 mM H₂O₂ solution
67 (996 µL H₂O + 6 µL H₂O₂, 50% 66 incubated for 30 minutes in a shaker incubator at 28°C at 200 rpm. A 100 mM H₂O₂ soluti
67 (996 µL H₂O + 6 µL H₂O₂, 50%) was then freshly prepared. 1 mM of H₂O₂ was then added
68 45 seconds as the culture incubated for 30 minutes in a shaker incubator at 28 6 at 200 rpm. A 100 mM H₂O₂ solution
68 45 seconds as the cultures were still shaking. The labeling experiment twas carried out
69 45 seconds as the cultures were s 68 45 seconds as the cultures were still shaking. The labeling experiment was carried out again with specific omissions as controls: no alkyne-phenol added, no H_2O_2 added, no alkyne-phenol and H_2O_2 added, differen 68 again with specific omissions as controls: no alkyne-phenol added, no H₂O₂ added, no alkyne-phenol and H₂O₂ added, no alkyne-phenol added, no H₂O₂ added, no alkyne-phenol added, no H₂O₂ added, no alkyne 70 alkyne-phenol and H_2O_2 added, different concentrations of alkyne-phenol added (0.5 m and 1 mM), and different H_2O_2 exposures (15 seconds, 30 seconds, and 60 seconds). 4 of cell culture were then quickly transfe 71 and 1 mM), and different H₃O₂ exposures (15 seconds, 30 seconds, and 60 seconds). 4 m

72 of cell culture were then quickly transferred into two 2 mL Eppendorf tubes and centrifuge

73 at 14,000 rpm for 10-15 secon 72 of cell culture were then quickly transferred into two 2 mL Eppendorf tubes and centrifuged
at 14,000 rpm for 10-15 seconds. The supernatant was quickly discarded, and the cell
pellets were resuspended in 1 mL of 65°C 37 at 14,000 rpm for 10-15 seconds. The supernatant was quickly discarded, and the cell

pellets were resuspended in 1 mL of 65°C pre-warmed TRIzol. Because proximity labeling

requires the folded APEX2 protein, its dena 74 pellets were resuspended in 1 mL of 65°C pre-warmed TRIzol. Because proximity label
requires the folded APEX2 protein, its denaturation in TRIzol can be used to quench the
proximity labeling reactions. RNA extraction f 24 pellets were resuspended in 1 mL of 65

75 requires the folded APEX2 protein, its de

proximity labeling reactions. RNA extrac

carried out. 55 μ L of a 10 mM Tris-HCl pl

air-dried RNA pellets. The samples were

tak enaturation in TRIzol can be used to quench the
tion followed by ethanol precipitation was then
H 7.0 (no EDTA) solution was added to dissolve the
vortexed briefly to aid resuspension. 5 µL were
ew tubes for Agilent Tapes 76 proximity labeling reactions. RNA extraction followed by ethanol precipitation was then
carried out. 55 µL of a 10 mM Tris-HCl pH 7.0 (no EDTA) solution was added to dissolve
air-dried RNA pellets. The samples were vor

77 carried out. 55 µL of a 10 mM Tris-HCl pH 7.0 (no EDTA) solution was added to dissolve t
air-dried RNA pellets. The samples were vortexed briefly to aid resuspension. 5 µL were
taken from the samples and placed in new air-dried RNA pellets. The samples were vortexed briefly to aid resuspension. 5 µL were
taken from the samples and placed in new tubes for Agilent Tapestation analyses and cell
lysate RNA sequencing.
Copper-catalyzed clic 79 taken from the samples and placed in new tubes for Agilent Tapestation analyses and compare Uysate RNA sequencing.
81 Copper-catalyzed click chemistry reactions ¹² were then conducted on the labeled RNA.
82 The react

80

80 Uysate RNA sequencing.

81 Copper-catalyzed click chemistry reactions ¹² were then conducted on the labeled RNA.

82 The reactions have a 300 μL total volume and contained: 50 μL of 100 ng/uL RNA (5 μg) +

83 17 81 Copper-catalyzed click of
82 The reactions have a 300
83 175 μ L H₂O + 12 μ L of 12
84 mix of 2.5 mM Cu(II)SO₄
85 reagents were added in 1
86 seconds and incubated
87 Afterward, RNA precipita Copper-catalyzed click chemistry reactions "- were then conducted on the labeled KNA.

82 The reactions have a 300 µL total volume and contained: 50 µL of 100 ng/uL RNA (5 µg) +

83 175 µL H₂O + 12 µL of 125 mM sodium a

83 $175 \mu L H_2O + 12 \mu L$ of 125 mM sodium ascorbate + 3 μL of 10 mM Cy5-Azide + 60 μL of a mix of 2.5 mM Cu(II)SO₄/12.5 mM THPTA (tris-hydroxypropyltriazolylmethylamine). The reagents were added in the order they ar mix of 2.5 mM Cu(II)SO₄/12.5 mM THPTA (tris-hydroxypropyltriazolylmethylamine). The
reagents were added in the order they are listed. The click reaction was vortexed for 5
seconds and incubated for 10 minutes at room te

France Condition of 2.5 mix of 2.5 mix of 2.5 mix of 2.5 mix of 2.5 magnets were added in the order they are listed. The click reaction was vortexed for 5 seconds and incubated for 10 minutes at room temperature away from seconds and incubated for 10 minutes at room temperature away from light exposure
Afterward, RNA precipitation was conducted and the RNA pellets were resuspended in
7

87 Afterward, RNA precipitation was conducted and the RNA pellets were resuspended in $\frac{8}{7}$

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- 89 **EXECUTE 20 PERCOVATE 100 ENALGE 100 ENALGE 100 ENALGE 100 EVALGE 1**
- 88 blot filtration on a nylon membrane following the manual's instructions of BIO RAD's Bio

89 blot filtration on a nylon membrane following the manual's instructions of BIO RAD's Bio

80 bot* and Bio-Dot SF Microfiltrat 91 SSC solution for at least 10 minutes. The pre-wetted nylon membrane was then assemble
92 into the dot blot apparatus. Vacuuming was applied to the apparatus and tighter sealing
93 was implemented. The wells were then w 92 into the dot blot apparatus. Vacuuming was applied to the apparatus and tighter sealing
93 was implemented. The wells were then washed 3 times with 1 mL of an ice-cold mixture of
94 10 mM NaOH/1 mM EDTA. Afterward, the 93 was implemented. The wells were then washed 3 times with 1 mL of an ice-cold mixture
94 10 mM NaOH/1 mM EDTA. Afterward, the 5 µg RNA samples were directly applied to the
95 wells (no RNA dissolution was performed). Th 994 10 mM NaOH/1 mM EDTA. Afterward, the 5 µg RNA samples were directly applied to the
95 wells (no RNA dissolution was performed). The wells were washed 3 times with 1 mL of an
96 ice-cold mixture of 10 mM NaOH/1 mM EDTA 95 wells (no RNA dissolution was performed). The wells were washed 3 times with 1 mL of ice-cold mixture of 10 mM NaOH/1 mM EDTA. The blotted membrane was washed 3 tim
10 for 5 minutes with a 30 mL solution containing 2X 96 ice-cold mixture of 10 mM NaOH/1 mM EDTA. The blotted membrane was washed 3 times
97 for 5 minutes with a 30 mL solution containing 2X SSC and 0.1% SDS. Fluorescent blot
98 inaging was then visualized using an ibright 97 for 5 minutes with a 30 mL solution containing 2X SSC and 0.1% SDS. Fluorescent blot
98 imaging was then visualized using an ibright imaging system at a fluorescent light exposure
99 of 50 ms under the Cy5 channel.
89 98 imaging was then visualized using an ibright imaging system at a fluorescent light expose of 50 ms under the Cy5 channel.

99 of 50 ms under the Cy5 channel.

90 RNase A & DNase I treatments

91 50 μ L of 100 ng/ μ 99 of 50 ms under the Cy5 channel.

89 of 50 ms under the Cy5 channel.

99 of 50 pL of 100 ng/pL of RNA collected from a proximity labeling reaction (4 mL of *Js767* cells

902 grown in M2G media with 2.5 mM alkyne phenol
- 105 the enzyme or mock treatments, the RNA was precipitated and resuspended in 50 μ L of 10
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- 101 50 μ L of 100 ng/ μ L of RNA colle

102 grown in M2G media with 2.5 m

103 either an RNase A (5 μ L of 10 m

104 treatment. The treated and mod

105 the enzyme or mock treatments

106 mM Tris-HCl p H 7.0 and the 102 grown in M2G media with 2.5 mM alkyne phenol. 45 seconds H₃O₂) were subjected to
102 grown in M2G media with 2.5 mM alkyne phenol. 45 seconds H₃O₂) were subjected to
103 either an RNase A (5 μL of 10 mg/mL RNa 102 grown in Masse A (5 µL of 10 mg/mL RNase A) or DNase I (5 µL of 1 U/µL DNase I)
104 treatment. The treated and mock samples were incubated for 2 hours at 37°C. Follow
105 the enzyme or mock treatments, the RNA was pre 104 treatment. The treated and mock samples were incubated for 2 hours at 37°C. Fol

105 the enzyme or mock treatments, the RNA was precipitated and resuspended in 5

106 mM Tris-HCl pH 7.0 and then subjected to the Coppe treatment. The treated and mock samples were incubated for 2 hours at 37°C. Following

the enzyme or mock treatments, the RNA was precipitated and resuspended in 50 µL of

106 mM Tris-HCl pH 7.0 and then subjected to the C 105 the ends of the ends of the ends of the ents of the Copper catalyzed click-chemistry reaction
107 with Cy5-azide. Following the 10-minute click reaction incubation, the RNA was then
105 with Cy5-azide. Following the 10 107 with Cy5-azide. Following the 10-minute click reaction incubation, the RNA was then
108 precipitated and subjected to dot blot filtration as previously described.
109 Streptavidin capture of biotin labeled RNA
10 To e 108 precipitated and subjected to dot blot filtration as previously described.

109 Streptavidin capture of biotin labeled RNA

10 To enrich the transcriptome of BR-bodies, RNA labeled with alkyne-phenol was subjected.

1 109 Streptart and subjected and subjected RNA

10 To enrich the transcriptome of BR-bodies, RNA labeled with alkyne-phen

11 to the following click chemistry reaction: 50 µL of RNA (total RNA volume

12 µL of 125 mM sodium 110 To enrich the transcriptome of BR-bodies,
111 to the following click chemistry reaction: 5
12 µL of 125 mM sodium ascorbate + 3 µL
113 2.5 mM Cu(II)SO₄/12.5 mM THPTA . The rea
114 click reaction was vortexed briefly 111 to the following click chemistry reaction: 50 μ L of RNA (total RNA volume) + 175 μ L H2O + 12 μ L of 125 mM sodium ascorbate + 3 μ L of 10 mM Biotin-peg3-azide + 60 μ L of a mix of 2.5 mM Cu(II)SO $u/12.5$ 112 12 12 U.com 112 12 12 U.com 12 0 11 M Notify 12 12 U.com 12 U.com 12 12 U.com 12 12 U.com 12 12 U.com 12.5 mM sodium ascorbate + 3 U.com 10 mM Biotin-peg3-azide + 60 U.L of a mix of
113 12.5 mM Cu(III)SO₄/12.5 mM TH 113 2.5 mM Cu(II)SO_a/12.5 mM THPTA . The reagents were added in order as they are listed. 1
114 click reaction was vortexed briefly and incubated for 10 minutes at room temperature aw
115 from light exposure. RNA precip 114 click reaction was vortexed briefly and incubated for 10 minutes at room temperature away
115 from light exposure. RNA precipitation was then conducted and the air-dried RNA pellets
116 were resuspended in 100 µL B&W b 115 from light exposure. RNA precipitation was then conducted and the air-dried RNA pellets
116 were resuspended in 100 µL B&W buffer (5 mM Tris-HCl, pH 7.5, 1 M NaCl, 0.5 mM EDTA,
117 0.1% v/v tween-20). The RNA samples w
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- 116 were resuspended in 100 µL B&W buffer (5 mM Tris-HCl, pH 7.5, 1 M NaCl, 0.5 mM EDTA,
117 0.1% v/v tween-20). The RNA samples were then immediately used for biotinylated RNA
118 enrichment.
119 High-capacity magnetic st 0.1% v/v tween-20). The RNA samples were then immediately used for biotinylated RNA

118 enrichment.

119 High-capacity magnetic streptavidin beads (Vectorslab) were washed three times with 1

120 mL B&W buffer, twice with 118 enrichment.

118 enrichment.

119 High-capacity magnetic streptavidin beads (Vectorslab) were washed three times with 1

120 mL B&W buffer, twice with 1 mL of a mix of 0.1 M NaOH/0.05 M NaCl, and once with 1 m

121 0.1 119 High-capacit
120 mL B&W buff
121 0.1 M NaCl a
122 mg/mL BSA +
- 120 mL B&W buffer, twice with 1 mL of a mix of 0.1 M NaOH/0.05 M NaCl, and once with 1 ml
121 0.1 M NaCl at room temperature. The beads were then blocked using a blocking buffer (1
122 mg/mL BSA + 1 mg/ml heparin salt, di
- 121 0.1 M NaCl at room temperature. The beads were then blocked using a blocking buffer (1
122 mg/mL BSA + 1 mg/ml heparin salt, dissolved in B&W buffer) at room temperature for 2
8
- 122 mg/mL BSA + 1 mg/ml heparin salt, dissolved in B&W buffer) at room temperature for 2
8 122 mg/mL BSA + 1 mg/ml heparin salt, dissolved in B&W buffer) at room temperature for 2
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- 124 vortexing. Afterward, the 100 μ L RNA solution was added to the beads along with an exercise 100 μ L of B&W buffer. The RNA and beads were allowed to mix at room temperature for one hour. The RNA-loaded beads were
- 125 100 µL of B&W buffer. The RNA and beads were allowed to mix at room temperature for
126 100 µL of B&W buffer. The RNA and beads were allowed to mix at room temperature for
126 with 1 mL of a PBS solution containing 4 126 one hour. The RNA-loaded beads were washed three times with 1 mL of B&W buffer, twith 1 mL of a PBS solution containing 4 M urea + 0.1% SDS, and twice with 1 mL of just

128 PBS at room temperature. To elute the enric 127 with 1 mL of a PBS solution containing 4 M urea + 0.1% SDS, and twice with 1 mL of just

128 PBS at room temperature. To elute the enriched RNA, 900 uL of 65°C pre-warmed TRIzol

129 was added to the beads and the sam 128 PBS at room temperature. To elute the enriched RNA, 900 uL of 65°C pre-warmed TRIzol

129 was added to the beads and the samples were incubated at 65°C for 10 minutes.

130 Afterward, 200 µL of chloroform was added an PBS at room temperature. To elute the enriched RNA, 900 uL of 65 ¹² pre-warmed TRIzot

28 was added to the beads and the samples were incubated at 65 °C for 10 minutes.

130 Afterward, 200 µL of chloroform was added and was added to the beads and the samples were included at 65 °C for 10 minutes.

Afterward, 200 µL 6 chloroform was added and the samples were inverted 6-7 time

131 then were incubated for 5 minutes at room temperature. Th 131 then were incubated for 5 minutes at room temperature. The samples were centrifuged at 14,000 rpm for 10 minutes at 4°C. The upper aqueous layer was transferred to a new Eppendorf tube and the enriched RNA was precipi 132 14,000 rpm for 10 minutes at 4⁹

133 Eppendorf tube and the enriche

134 dried RNA pellets were resuspe

135 µL were then used for Agilent Ta

136 for the follow-up RNA-seq expe

137 RNA quality assessment

138 *Is* d RNA was precipitated as previously described. The
nded in 10 µL of 10 mM Tris-HCl pH 7.0 and 0.1 mM E
pestation analysis. The remaining volume could be u
riments.
frown in PYE at 28°C overnight and re-inoculated into
tu 137 RNA quality assessment

138 Js767 (RNE-Apex2FlgC-2) was grown in F

139 M2G the next day. Once the cultures rea

140 added and incubated for 30 minutes in a

141 H₂O₂ was added to the culture for 45 sec

142 preci 138 *Js767 (RNE-Apex2FlgC-2)*

139 M2G the next day. Once t

140 added and incubated for

141 H₂O₂ was added to the cu

142 precipitated from 4 mL of

143 100 ng/µL of alkylated RN

chemistry reaction: 50 µL

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- 14.000 rpm for 10 minutes at 4°C. The upper aqueous layer was transferred to a new

13.3 thepend of tube and the enriched RNA was precipitated as previously described. The air-

131 dried RNA pellets were resuspended in 1 135 plt were then used for Agilent Tapestation analysis. The remaining volume could be utilized
136 plt were then used for Agilent Tapestation analysis. The remaining volume could be utilized
136 for the follow-up RNA-seq 135 135 11 m 100 m M Communication and the metallom and the brief of the clubs
135 138 157 (RNE-Apex2FIgC-2) was grown in PYE at 28°C overnight and re-inoculated into 5 mL
139 125 the next day. Once the cultures reached an Js8

JS8 M2C (*RNE-Apex2FlgC-2)* was grown in PYE at 28°C overnight and re-inoculated into 5 mL

139 M2G the next day. Once the cultures reached an OD of 1.2, 2.5 mM of alkyne-phenol was

426 ded and incubated for 30 minu
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- 149 Iris-HCl pH 7.0 and 0.1 mM EDTA. 250 ng of RNA from the Js 767 RNA lysate and the click
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- 140

140 added and incubated for 30 minutes in a shaker incubator at 28°C at 200 rpm. 1 mM of

141 H_2O_2 was added to the culture for 45 seconds. Total RNA was then collected and

142 precipitated from 4 mL of culture added and the culture of 3 minutes in a shaker incubator at 28 °C at 200 rpm. 1 mM
142 precipitated from 4 mL of culture as previously described using TRIzol. Afterward, 50 µ1
143 precipitated from 4 mL of culture as previ 142 ris-HCl pH 7.0 and 0.1 mM EDTA. 250 ng of RNA from the *S2C* researched and 1 mL of culture as previously described using TRIzol. Afterward
143 100 ng/µL of alkylated RNA were subjected to the following copper catalyz 143 100 ng/µ of alkylated RNA were subjected to the following copper catalyzed click-

chemistry reaction: 50 µL of 100 ng/µL RNA (5 µg) + 175 µL H₂O + 12 µL of 125 mM sodium

ascorbate + 3 µL of 10 mM Cy5-Azide + 15 µL 145 ascorbate + 3 μ L of 10 mM Cy5-Azide + 15 μ L of 100 mM aminoguanidine hydrochloric acidem 146 μ L of 10 mM cV₅-Azide + 15 μ L of 100 mM aminoguanidine hydrochloric acidem 146 16 10 minutes at room temperatu 146 $+60 \mu$ L of a mix of 2.5 mM Cu(II)SO₄/12.5 mM THPTA. The click reaction was incubated for
147 10 minutes at room temperature away from light exposure. The RNA from the click reaction
148 origins at room temperature 147 10 minutes at room temperature away from light exposure. The RNA from the click reaction
148 10 minutes at room temperature away from light exposure. The RNA from the click reaction
149 11:5 HCl pH 7.0 and 0.1 mM EDTA 148 was then precipitated and the air-dried RNA pellet was resuspended in 50 μ L of 10 mM
149 Tris-HCl pH 7.0 and 0.1 mM EDTA. 250 ng of RNA from the *ls* 767 RNA lysate and the click
150 reaction RNA were sent for Agil 149 Tris-HCI pH 7.0 and 0.1 mM EDTA. 250 ng of RNA from the Js767 RNA lysate and the clice
150 reaction RNA were sent for Agilent Tapestation analysis.
151 RNA precipitation
152 RNA precipitation
152 RNA precipitation
153 149 Tris-Hotel Privarius C. Find C. Excellent Tapestation analysis.

149 Tris-Hotel phenomenology and the Color reaction RNA precipitation

152 RNA precipitation

152 RNA precipitation was carried out by adding, in order, **EXEMPLE PRONON RNA precipitation**

151 **RNA precipitation**

152 **RNA precipitation was carried out by adding, in order, 2** μ **

153 sample volume of 3 M sodium acetate pH 5.5, and 1 sam

154 sample mixtures were vigoro** 151 RNA precipitation
152 RNA precipitation v
153 sample volume of 3
154 sample mixtures we
155 spun for 1 hour at 1
156 80% ethanol was pipette
157 ethanol was pipette 153 sample volume of 3 M sodium acetate pH 5.5, and 1 sample volume of isopropanol. T
154 sample mixtures were vigorously vortexed and stored at -80°C overnight. The samples
155 spun for 1 hour at 14,000 rpm. The supernat 154 sample mixtures were vigorously vortexed and stored at -80°C overnight. The samples were spun for 1 hour at 14,000 rpm. The supernatant was then decanted and 1 mL of ice-cold 80% ethanol was added. The samples were sp sample mixtures were vigorously vortexed and stored at -80°C overnight. The samples were
spun for 1 hour at 14,000 rpm. The supernatant was then decanted and 1 mL of ice-cold
80% ethanol was added. The samples were spun fo
-
- 156 80% ethanol was added. The samples were spun for 15 minutes at 14,000 rpm. The
157 ethanol was pipetted out and the samples were then spun for 1 minute at 14,000 rpm. And
9 ethanol was pipetted out and the samples were then spun for 1 minute at 14,000 rpm.
9 157 ethanol was pipetted out and the samples were then spun for 1 minute at 14,000 rpm. Any
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- 159 became translucent. The RNA was then resuspended in the buffer of choice.

158 became translucent. The RNA was then resuspended in the buffer of choice.

161 The following buffers were initially prepared:

162 1) 10X 169 Western Blot

169 Western Blot

169 The following buffers were initially prepared:

162 1) 10X transfering buffer: 144 grams of glycine and 30.2 grams of Tris-Bas

163 dissolved in 900 mL of ddH2O. The buffer volume wa 161 The following H

162 1) 10X trains

163 dissolv

164 then filt

165 2) 10X TBS

166 ddH2O

167 buffer v

168 Rapid-f

169 3) 1X TBS

170 was the

171 *NA1000, LS43*

171 *NA1000, LS43*

172 incubated ove

173 from each 162 1) 10X transfering buffer: 144 grams of gl
163 dissolved in 900 mL of ddH2O. The bu
164 then filtered utilizing a Nalgene Rapid-
165 2) 10X TBS: 24 grams of Tris-Base and 88
166 ddH2O. The pH of the buffer was adju-
1 163 dissolved in 900 mL of ddH2O. The buffer volume was adjusted to 1 L of ddH2

163 dissolved in 900 mL of ddH2O. The buffer volume was adjusted to 1 L of ddH2

164 then filtered utilizing a Nalgene Rapid-flow sterile di 164 then filtered utilizing a Nalgene Rapid-flow sterile disposable filter unit.

165 2) 10X TBS: 24 grams of Tris-Base and 88 grams of NaCl were dissolved in 900 mL of

166 ddH2O. The pH of the buffer was adjusted to 7.6 165 2) 10X TBS: 24 grams of Tris-Base and 88 grams of NaCl were dissolved in the differed validary of the buffer was adjusted to 7.6 utilizing hydrochloric a
167 buffer volume was adjusted to 1 L of ddH2O and then sterili dH2O. The pHof the buffer was adjusted to 7.6 utilizing hydrochloric acid. The

167 buffer volume was adjusted to 1 L of ddH2O and then sterilized utilizing a Nalgene

Rapid-flow sterile disposable filter unit.

3) 1X TBST 167 buffer volume was adjusted to 1 L of ddH2O and then sterilized utilizing a Nalger

168 Rapid-flow sterile disposable filter unit.

169 SIMEST: 10X TBS was diluted 10-fold with ddH2O to make 1X TBS. 0.1% Tween-

170 wa
- 168 Rapid-flow sterile disposable filter unit.

169 3) 1X TBST: 10X TBS was diluted 10-fold with ddH2O to make 1X TBS. 0.1% Tween-20

170 was then added to the 1X TBS solution to make 1X TBST.

171 NA1000, LS4379 (hfq-m2) 169 3) 1X TBST: 10X TBS was diluted 10-fold with
170 was then added to the 1X TBS solution to
171 NA1000, LS4379 (hfq-m2), and Js767(RNE-Ape;
172 incubated overnight at 28°C until they reached
173 from each culture, place

- 170 was then added to the 1X TBS solution to make 1X TBST.

171 NA1000, LS4379 (hfq-m2), and Js767(RNE-Apex2FlgC-2) were inoculated in PYE and

172 incubated overnight at 28°C until they reached log phase (OD~0.3-0.6). 1 MA1000, LS4379 (hfq-m2), and Js767(RNE-Apex2FIgC-2) were in
172 incubated overnight at 28°C until they reached log phase (OD ~ (from each culture, placed in 1.5 mL Eppendorf tubes, and centr
174 6,000 rpm. The liquid cultu 171 Noticoty, Lawy Since the United by transformation and Calculated ont The and the PVDF membrane was proported in 1.5 mL Eppendoff tubes, and centrifuged for 2 minutes from each culture, placed in 1.5 mL Eppendoff tubes incubated overnight at 28°C until they reached log phase (OD ~0.3-0.6). I mL was taken
173 from each culture, placed in 1.5 mL Eppendoff tubes, and centifityed for 2 minutes at
174 6,000 rpm. The liquid cultures were disc
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- 174 6,000 rpm. The liquid cultures were discarded, and the cells were resuspended in 4X 5 loading dye. 125 µL of 4X SDS loading dye was added to cultures with an OD~0.5. The samples were boiled at 95 °C for 5 minutes then 175 loading dye. 125 μ of 4X SDS loading dye was added to cultures with an OD-0.5. The
176 samples were boiled at 95 °C for 5 minutes then vortexed vigorously. The vortexed sample:
177 were quickly spun down and placed 176 samples were boiled at 95°C for 5 minutes then vortexed vigorously. The vortexed samples were quickly spun down and placed on ice. 5 µL of a pre-stained PageRuler marker and placed to the sample were loaded onto an SDS samples were boiled at 95 °C for 5 minutes then vortexed vigorously. The vortexed variances already and placed on ice. 5 µL of spre-stalined PageRuler maker and 20 µL of lysate from each sample were loaded onto an SDS-PAGE 178 μ L of lysate from each sample were loaded onto an SDS-PAGE gel. 1X transferring buffer
179 μ L of lysate from each sample were loaded onto an SDS-PAGE gel. 1X transferring buffer
179 while the samples were runnin 179 was freshly prepared (20 mL of 10X transfer buffer + 20 mL of methanol in 160 mL ddH2O

180 while the samples were running on the gel. A PVDF transferring membrane was wetted in

181 methanol and 6 blotting papers were While the samples were running on the gel. A PVDF transferring membrane was wetted in methanol and 6 blotting papers were wetted using 1X transferring buffer for at least 10 minutes. Once the lysate samples on the SDS-PAG 181 methanol and 6 blotting papers were wetted using 1X transferring buffer for at least 10
182 minutes. Once the lysate samples on the SDS-PAGE were properly resolved, the gel was
183 placed on top of 3 pre-wetted blottin 182 minutes. Once the lysate samples on the SDS-PAGE were properly resolved, the gel was placed on top of 3 pre-wetted blotting papers, and 2 mL of 1X transferring buffer was poured on top of 3 pre-wetted blotting papers, 183 placed on top of 3 pre-wetted blotting papers, and 2 mL of 1X transferring buffer was
184 poured on the gel. The PVDF membrane was briefly plunged into the 1X transferring buffer
185 and then placed on top of the gel.
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- 184 poured on the gel. The PVDF membrane was briefly plunged into the 1X transferring b
185 and then placed on top of the gel. The remaining 3 blotting papers were placed on top
186 the PVDF membrane. The transferring of t 185 and then placed on top of the gel. The remaining 3 blotting papers were placed on top of the PVDF membrane. The transferring of the lysates from the SDS-PAGE gel to the PVDF membrane occurred by utilizing the BIO-RAD T 186 the PVDF membrane. The transferring of the lysates from the SDS-PAGE gel. to the PVDF
187 membrane occurred by utilizing the BIO-RAD Trans-Blot Turbo Transfer system (1 amps,
188 2.5 volts, 15 minutes). Once the transf membrane occurred by utilizing the BIO-RAD Trans-Blot Turbo Transfer system (1 amps,
188 2.5 volts, 15 minutes). Once the transfer was completed, the PVDF membrane was place
189 in a new container containing 5% BSA (blocki
- 2.5 volts, 15 minutes). Once the transfer was completed, the PVDF membrane was place
in a new container containing 5% BSA (blocking solution). The container was nutated for
190 in a new container containing 5% BSA (blockin
- in a new container containing 5% BSA (blocking solution). The container was nutated for 1
188 in a new container containing 5% BSA (blocking solution). The container was nutated for 1
190 hour at room temperature. Afterwar
- 190 hour at room temperature. Afterward, the 5% BSA blocking solution was discarded and the
191 PVDF membrane was washed 5 times with ddH2O for 5 minutes per wash. The primary
192 antibody (DYKDDDDK anti-flag tag) was then
-
-
- 191 PVDF membrane was washed 5 times with dd H2O for 5 minutes per wash. The primary
192 antibody (DYKDDDDK anti-flag tag) was then diluted (1:10000) into a fresh 5% BSA
193 solution. Once the last dd H2O wash was complete 192 antibody (DYKDDDDK anti-flag tag) was then diluted (1:10000) into a fresh 5% BSA
193 solution. Once the last ddH2O wash was completed, the primary antibody solution was
10 193 solution. Once the last ddH2O wash was completed, the primary antibody solution
10 $\frac{1}{2}$ solution. Once the primary and primary and

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195 following day, the primary antibody solution was discarded and the F

196 washed 5 times with TBST for 5 minutes per wash. The secondary and

196 washed 5 times with TBST for 5 minutes per wash. The secondary

19 PVDF membrane
tibody (goat anti-
o a fresh 5% BSA
tibody solution w
om temperature
nen discarded an
ash. Following the
was prepared (1
The PVDF membred on top of the
room temperatu
nto an iBright ima
28°C. The overnig
e ce 197 secondary antibody, HRP conjugated) was then diluted (1:10000) into a fresh 5% BSA

198 solution. Once the last TBST wash was completed, the secondary antibody solution was

199 poured on the PVDF membrane, and the co 200 bour avoiding light exposure. The secondary antibody solution was then discarded and the

201 bour avoiding light exposure. The secondary antibody solution was then discarded and the

202 was placed on Seran wap and t
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- 196 washed 5 times with TBST for 5 minutes per wash. The secondary antibody (goat anti-rabb
197 secondary antibody, HRP conjugated) was then diluted (1:10000) into a fresh 5% BSA
198 solution. Once the last TBST wash was 1988 solution. Once the last TBST wash was completed, the secondary antibody solution was poured on the PVDF membrane, and the container was nutated at room temperature 10

200 hour avoiding light exposure. The secondary 199 poured on the PVDF membrane, and the container was nutated at room temperature for

200 hour avoiding light exposure. The secondary antibody solution was then discarded and the PVDF membrane was washed 5 times with TB
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201 PVDF membrane was washed 5 times with TBST for 5 minutes per wash. Following the final

202 washing procedure, a pierce ECL western blotting substrate solution was prepared (1 mL

203 of reagent 1 + 1 mL of reagent 2 202 **Example 2012** Washing procedure, a pierce ECL western blotting substrate solution was prepared (1 mL

2023 washing procedure, a pierce ECL western blotting substrate solution was prepared (1 mL

2043 of reagent 1 + 1 202 of reagent 1 + 1 mL of reagent 2 were mixed and vigorously vortexed). The PVDF membrane

204 of reagent 1 + 1 mL of reagent 2 were mixed and vigorously vortexed). The PVDF membrane

204 was placed on Seram wrap and th 204 was placed on Seram wrap and the 2 mL substrate solution was poured on top of the

205 membrane. The substrate was incubated on the PVDF membrane at room temperature for

2016 5 minutes avoiding light exposure. The me 205 membrane. The substrate was incubated on the PVDF membrane at room temperature 3 minutes avoiding light exposure. The membrane was then placed into an iBrightima
2023 Sminutes avoiding light exposure. The membrane was 205 Sminutes avoiding light exposure. The membrane was then placed into an iBright imaging

207 system and the signal was detected under the Chemi-blot filter.

208 M41000 & Js767 (RNE-Apex2FIgC-2) were grown in PYE overn 206 206 minutes avoiding light exposure. The membrane was the membrane was the membrane was the membrane was detected under the Chemi-blot filter.

208 M41000 & Js767 (RNE-Apex2FIgC-2) were grown in PYE overnight at 28°C. 208 Dilution Plates

209 M41000 & Js767 (RNE-Apex2FIgC-2) were grown in PYE overnight

210 cultures were diluted into fresh media and incubated at 28°C unit

211 of ~0.3-0.6. Cells were then diluted to an OD=0.05 in PYE a 208 Didden rates

209 NA1000 & Js767

210 cultures were di

211 of ~0.3-0.6. Cell

212 were made. 5 µL

213 incubated at 28°

215 Js87 (RNE-msfG

215 Js87 (RNE-msfG

215 Js87 (RNE-msfG

217 1.5% agarose pa

219 MYO-CCD ca NA1000 & Js767 (RNE-Apex2FigC-2) were grown in PYE overnight at 28°C

210 cultures were diluted into fresh media and incubated at 28°C until the c

211 of ~0.3-0.6. Cells were then diluted to an OD=0.05 in PYE and 4 10-fo Example the serial dilutions
plates were
imaging system.
PYE + gent (0.5
fixed on M2G +
cientific). Nikon
with a CoolSNAP
pture the images
covernight. Cells
overnight. Cells
arnight log phase
d to an OD = 0.05
ls were adde

cultures were unule unto fresh media and incubated at 28^{-C} untuit the cells reached at 28^{-C} for two days, and images were taken using an ibright imaging system.

211 of ~0.3-0.6. Cells were the riducted to an OD=0.05 i 221 were made. 5 µL were spotted from each dilution onto PYE plates. The plates were

212 were made. 5 µL were spotted from each dilution onto PYE plates. The plates were

214 **Fluorescent Cell Imaging**

214 **Fluorescent** 213 incubated at 28°C for two days, and images were taken using an ibright imaging sys

214 **Fluorescent Cell Imaging**

215 *Is87 (RNE-msfGFP) & Js768 (RNE-Apex2FIgC-EGFPC-2)* were grown in PYE + gent (

216 μ g/mL) and 213 Incubated at 28°C for two days, and images were taken using an ibright imaging system.

214 Eluorescent Cell Imaging

215 Ista 7 (RNE-msfGFP) & Is 768 (RNE-Apex2FigC-EGFPC-2) were grown in PYE + gent (0.5

216 µg/mL) 214 Fluorescent Cell Imaging

215 Js87 (RNE-msfGFP) & Js768

216 µg/mL) and PYE + Kan, resp

217 1.5% agarose pads placed

219 MYO-CCD camera and a 10

220 mRNA half-lives measure

222 MA1000 and Js767 (RNE-Ag

223 were t 215 Jstephend PYE + Kan, respectively, overnight at 28°C. Cells were then fixed on M2G + 1.5% agarose pads placed on microscope slides (3051, Thermofisher scientific). Nikon elements software was used to control a Nikon Ec 216 BymL) and PYE + Kan, respectively, overnight at 28°

217 1.5% agarose pads placed on microscope slides (3C

218 elements software was used to control a Nikon Eclip

MYO-CCD camera and a 100x Oil CFI Plan Fluor (Nik

2 151, Thermofisher scientific). Nikor
161, Thermofisher scientific). Nikor
1698 - NI-E equipped with a CoolSNA
1699) objective to capture the image
161
161 - Incubated until overnight log phas
161 - 161 - 161 - 161 - 161 -218 elements software was used to control a Nikon Eclipse NI-E equipped with a CoolSNAP
219 elements software was used to control a Nikon Eclipse NI-E equipped with a CoolSNAP
220 mRNA half-lives measurement
221 mRNA half-219 MYO-CCD camera and a 100x Oil CFI Plan Fluor (Nikon) objective to capture the images

220 mRNA half-lives measurement

222 MA1000 and Js767 (RNE-Apex2FIgC-2) were grown in liquid PYE at 28°C overnight. Cells

223 were 220

221 MRNA half-lives measurement

222 MA1000 and Js767 (RNE-Apex2FigC-2) were grown in liquid PYE at 28°C overnight. Cells

223 were then serially re-inoculated into liquid M2G and incubated until overnight log phase
 220 221 mm/M had-dves measurement

222 MA1000 and Js 767 (RNE-Apex2Fl,

223 were then serially re-inoculated i

224 (OD ~0.3-0.6) was reached. Log p

225 25 mL liquid M2G. Before adding

226 mL of RNAprotect Bacterial reage
 NATIOUD and JS767 (RNE-Apex2FigC-2) were grown in iquid PYE at 28 °C overnight. Cells

223 were then serially re-inoculated into liquid M2G and incubated until overnight log phase

224 (OD ~0.3-0.6) was reached. Log phase 224 (OD ~0.3-0.6) was reached. Log phase cultures were then re-inoculated to an OD = 0.05
225 25 mL liquid M2G. Before adding rifampicin, at time point 0, 1 mL of cells were added to .
226 mL of RNAprotect Bacterial reage 225 25 mL liquid M2G. Before adding rifampicin, at time point 0, 1 mL of cells were added to 2
226 mL of RNAprotect Bacterial reagent (Qiagen) and vortexed for 5 seconds. 200 µg/mL of
227 Rifampicin was administered to th 226 mL of RNAprotect Bacterial reagent (Qiagen) and vortexed for 5 seconds. 200 µg/mL of
227 Rifampicin was administered to the cultures and 1 mL of cells were extracted and added t
228 2 mL of RNAprotect Bacterial reagent 227 Rifampicin was administered to the cultures and 1 mL of cells were extracted and adde
228 2 mL of RNAprotect Bacterial reagent at each of the following time points (followed by 5
329 seconds vortexing): 1, 2, 4, and 8 228 2 mL of RNAprotect Bacterial reagent at each of the following time points (followed by 5
229 seconds vortexing): 1, 2, 4, and 8 minutes. Cells were incubated at room temperature in
11 229 seconds vortexing): 1, 2, 4, and 8 minutes. Cells were incubated at room temperature in
 11 229 seconds vortexing): 1, $2, 4, 4, 5, ...$

231 minutes. The bacterial pellets were resuspended in 1 mL of 65^oC pre-heated TRizol

231 minutes. The bacterial pellets were resuspended in 1 mL of 65^oC pre-heated TRizol

232 (Ambion) and incubated at 65^oC for 10 minutes. The bacterial pellets were resuspended in 1 mL of 65 °C pre-heated TRizol
2012 (Ambion) and inclubated at 55°C for 10 minutes. 200 µL of chloroform was then add
2012 the samples were inclubated at foom temperature 232 (Ambion) and incubated at 65°

233 the samples were incubated at 65°

234 samples were centrifuged at 1

235 removed and placed in a new

236 the pellets were resuspended

237 EDTA). PCR tubes were filled w

238 prime t room temperature for 5 minutes. Subsequently, the
4,000 rpm for 10 minutes at 4°C. The aqueous layer was
1.5 mL Eppendorf tube. The RNA was then precipitated and
in 50 µL elution buffer (10 mM Tris-HCl, pH=7.0, 0.1 mM
i 234 samples were centrifuged at 14,000 rpm for 10 minutes at 4°C. The aqueous layer were removed and placed in a new 1.5 mL Eppendorf tube. The RNA was then precipitate

235 removed and placed in a new 1.5 mL Eppendorf tu samples were centrifuged at 14,000 rpm for 10 minutes at 4 °C. The aqueous layer was

236 removed and placed in a new 1.5 mL Eppendoff tube. The RNA was then precipiteted a

236 the pellets were resuspended in 50 µL eluti 236 the pellets were resuspended in 50 μ L elution buffer (10 mM Tris-HCl, pH=7.0, 0.1 mM
237 EDTA). PCR tubes were filled with a master mix that contained 0.4 μ M of ctrA forward
238 primer and 0.4 μ M of ctrA reve 237 EDTA). PCR tubes were filled with a master mix that contained 0.4 µM of ctrA forward
238 primer and 0.4 µM of ctrA reverse primer, 1X Luna Universal One-Step Reaction Mix, 1X
239 Luna WarmStart RT Enzyme Mix, and wate 228

228 Eprimer and 0.4 µM of ctrA reverse primer, 1X Luna Universal One-Step Reaction Mix, 1

238 Eprimer and 0.4 µM of ctrA reverse primer, 1X Luna Universal One-Step Reaction Mix, 1

239 Luna WarmStart RT Enzyme Mix, 238 primer and 0.4 princreative course primers, 1x Canar One-Step Interests concervences primers and water. 100 ng/pL of RNA template was additionally aliquoted into each of the PCR tubes. The samples were mixed well and 240 aliquoted into each of the PCR tubes. The samples were mixed well and quickly spun
241 down. A QuantStudio Real-Time PCR apparatus was utilized to conduct and examine the
242 gRT-PCR experiments. The same qRT-PCR expe 241 alia down. A QuantStudio Real-Time PCR apparatus was utilized to conduct and examine
242 alient-PCR experiments. The same qRT-PCR experiment was done using instead the 5S
243 forward and reverse primers. To determine 242 down. A must be then converted into RNA-decay rates, we fitted a linear curve

242 dRT-PCR experiments. The same qRT-PCR experiment was done using instead the SS

243 forward and reverse primers. To determine the mRNA 242 Toward and reverse primers. To determine the mRNA-decay rates, we fitted a linear content to the ln (fraction RNA remaining) at each time point. Using a standard curve, the Ct word converted into the quantity of RNA. 244 to the In (fraction RNA remaining) at each time point. Using a standard curve, the Ct was

245 converted into the quantity of RNA. Each time point's 5S rRNA amount was divided by the

246 amount of SS rRNA at time poi

converted into the quantity of RNA. Each time point's 5S rRNA amount was divided by the

245 amount of 5S rRNA at time point 0. The number obtained at each time point was then use

247 to normalize the amount of ctrA at ea converted into the quantity of RNA. Each time point's 5S rRNA amount was divided by the

amount of 5S rRNA at time point 0. The number obtained at each time point was then used

to normalize the amount of ctark at each tim 247 to normalize the amount of ctrA at each time point. The natural log of % RNA remaining
248 found in each sample was divided by the natural log of RNA at time point 0. The slopes of
249 the linear curve fit were then co 248

248 found in each sample was divided by the natural log of RNA at time point 0. The slopes of

249 the linear curve fit were then converted into mRNA half-life using the following equation

250 mRNA half-life=-ln(2)/ 249 the linear curve fit were then converted into mRNA half-life using the following equation:

250 mRNA half-life=-ln(2)/slope.

251 **Plasmid Construction**

252 pAPEX2-ElgC-2 Kan^R.

253 The pFlgC-2 vector was PCR ampli 250 mRNA half-life=-ln(2)/slope.

251 **Plasmid Construction**

252 pAPEX2-FigC-2 tector was PCR amplified using primers HY1F & HY1R. APEX2 was PCR

254 amplified using primers HY2F & HY2R from the addgene template #129640. 251 **Plasmid Construction**

252 pAPEX2-FIgC-2 Kan^R

253 The pFIgC-2 vector was PCR a

254 amplified using primers HY2F

255 were ran on a 1% agarose gel

256 purified vector was Dpn1 trea

257 Kit. The Apex2FIgC-2 Kan^R 251 Prasmid Construction
252 pAPEX2-FIgC-2 Kan^R
253 The pFIgC-2 vector was
254 amplified using primers
255 were ran on a 1% agaros
256 purified vector was Dpn
257 Kit. The Apex2FIgC-2 Ka
259 pg/mL) plates. The resu
260 252 pAPEX2-FigC-2 Kan

253 The pFlgC-2 vector w

254 amplified using prime

were ran on a 1% age

256 purified vector was D

257 Kit. The Apex2FlgC-2

259 transformed into che

269 pg/mL) plates. The re

260 Miniprep Kit 254 amplified using primers HY2F & HY2R from the addgene template #129640. The amplified using primers HY2F & HY2R from the addgene template #129640. The amplified vector was Dpn1 treated then column purified using the Ge 255 were ran on a 1% agarose gel and gel extracted using a GeneJET Gel Extraction Kit. The

256 purified vector was Dpn1 treated then column purified using the GeneJET PCR Purification

257 Kit. The Apex2FigC-2 *Kan*^R p 256 purified vector was Dpn1 treated then column purified using the GeneJET PCR Purificat

257 Kit. The Apex2FigC-2 Kan^R plasmid was assembled via Gibson assembly (NEB) and

258 transformed into chemically competent DHb 257 Kit. The Apex2FigC-2 *Kan*^R plasmid was assembled via Gibson assembly (NEB) and
258 transformed into chemically competent DHbeta10 *E*. coli and plated on LB + Kan (50
259 pg/mL) plates. The resulting KanR colonies 257 KIt. The Apex2FigC-2 Kan plasmid was assembled via Gibson assembly (NEB) and

258 transformed into chemically competent DHbeta10 *E. coli* and plated on LB + Kan (5

259 pg/mL) plates. The resulting KanR colonies were 259 transformed into chemically competent DHbeta10 E. coli and plated on LB + Kan (50
259 transformed into chemically competent DHbeta10 E. coli and plated on LB + Kan (50
260 Miniprep Kit and screened via restriction dig 260 Miniprep Kit and screened via restriction digestion (EcoR1) and the insert sequence was
verified by Sanger sequencing (Genewiz).
262 pRNE-Apex2-FigC-2 Kan^R
262 pRNE-Apex2-FigC-2 Kan^R

- 261 werified by Sanger sequencing (Genewiz).
262 DRNE-Apex2-FigC-2 Kan^R
262 DRNE-Apex2-FigC-2 Kan^R
- 262 pRNE-Apex2-FigC-2 Kan^R 262 pRNE-Apex2-FigC-2 Kan
-
- The last 534 RNase E (RNE) base pairs were obtained by digesting pRNE-YFPC-1 11 With
264 Mde1 and Kpn1. APEX2FIgC-2 was digested with Nde1 and Kpn1. The digestion reaction
265 were ran on a 1% agarose gel and the RNE fragm
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- 266 extracted using a GeneJET Gel Extraction Kit. The RNE fragment and Apex2FlgC-2 vector
267 were ligated using T4 ligase, transformed into chemically competent DHbeta10 *E. coli*
268 selected on LB + Kan plates. The res 267 extraction Kit. The purified using primers were minipreped using the Sectred on LB + Kan plates. The resulting KanR colonies were minipreped using the GeneJET Plasmid Miniprep Kit and screened via restriction digestio 268 SenelET Plasmid Miniprep Kit and screened via restriction digestion (EcoRV) and the sequence was verified by Sanger sequencing (Genewiz).

270 sequence was verified by Sanger sequencing (Genewiz).

271 pRNE-Apex2FIgC-
- 265 were ran on a 1% agarose gel and the RNE fragment and Apex2-FigC-2 vector were gel

266 extracted using a GeneJET Gel Extraction Kit. The RNE fragment and Apex2FigC-2 vector were ligated using T4 ligase, transformed i
-
- ^{Were} were valuated using in the agacy, ransformed into chemically completed using the

268 selected on LB + Kan plates. The resulting KanR colonies were minipreped using the

269 GenelET Plasmid Miniprep Kit and screene 270 sequence was verified by Sanger sequencing (Genewiz).

271 pRNE-Apex2FIgC-EGFPC-2 Kan^R

272 The RNE-Apex2FIgC-2 vector was PCR amplified using primers HY3F & HY3R. The EGFP

273 insert was PCR amplified using primer
-

Js767: NA1000 rne::rne-apex2-flg KanR

-
- 271 pRNE-Apex2FlgC-EGFPC-2 Kan^R
272 The RNE-Apex2FlgC-EGFPC-2 Kan^R
273 insert was PCR amplified using primers HY4F & HY4R fro
274 The amplicons were ran on a 1% agarose gel and gel extr.
275 Extraction Kit. The purifi
- 271 pRNE-Apex2FlgC-EGFPC-2 Kan

272 The RNE-Apex2FlgC-2 vector was

273 insert was PCR amplified using p

274 The amplicons were ran on a 1%

275 Extraction Kit. The purified vector

276 GeneJET PCR Purification Kit. The
 272 Insert was PCR amplified using primers HY4F & HY4R from the addgene template #1296

274 Ine amplicons were ran on a 1% agarose gel and gel extracted using a GeneJET Gel

275 Extraction Kit. The purified vector was Dpn 274 The amplicons were ran on a 1% agarose get and get extracted using a GeneJET Get

275 Extraction Kit. The purification Kit. The RNE-Apex2FigC-EGFPC-2 plasmid was assembled via

277 Gibson assembly (NEB) and transforme 275 Extraction Kit. The purified vector was Dpn1 treated then column purified using the
276 GeneJET PCR Purification Kit. The RNE-Apex2FIgC-EGFPC-2 plasmid was assemble
277 Gibson assembly (NEB) and transformed into chemic 276 Extraction Kit. The RNE-Apex2FigC-EGFPC-2 plasmid was assembled vector was assembled vector of Debias and the purification Kit. The RNE-Apex2FigC-EGFPC-2 plasmid was assembled vector was verified by Sanger sequencial 277 Gibson assembly (NEB) and transformed into chemically competent DHbeta10 E. coli and selected on LB + Kan plates. The resulting KanR colonies were minipreped using the GeneJET Plasmid Miniprep Kit and screened via res 278 selected on LB + Kan plates. The resulting KanR colonies were minipreped using the

279 selected on LB + Kan plates. The resulting KanR colonies were minipreped using the

279 GeneJET Plasmid Miniprep Kit and screened 279 GeneJET Plasmid Miniprep Kit and screened via restriction digestion (EcoRV) and the
279 GeneJET Plasmid Miniprep Kit and screened via restriction digestion (EcoRV) and the
281 Strain construction
282 *Is 767: NA1000 m* 289 Sequence was verified by Sanger sequencing (Genewiz).

281 Strain construction

282 Strain construction

282 The RNE-Apex2FigC2 plasmid was recombined into the *me* locus in NA1000 via mating and

283 The RNE-Apex2Fig 281 Strain construction
282 *Is 767: NA1000 rne::rne-apex2-flg Kan^e*
283 The RNE-Apex2FlgC2 plasmid was recombined into the *r*
284 the selection was carried out on PYE + Nal (20 µg/mL) + K
285 resulting KanR colonies we 282 *Is767: NA1000 rne::r.*
283 The RNE-Apex2FlgC2
284 the selection was care
285 resulting KanR coloni
286 by PCR.
287 *Is768: NA1000 rne::r.*
288 The RNE-Apex2FlgC-
289 mating and the selec
290 were first grown in PY
291 283
284 285
286 7
288 289 291
292 292 284 The Relection was carried out on PYE + Nat (20 pg/mL) + Kan (25 pg/mL) plates. The resulting KanR colonies were first grown in PYE + Kan (5 pg/mL) cultures and then screened by PCR.

285 The RNE-Apex2FlgC-EGFPC-2 plas resulting KanR colonies were first grown in PYE + Kan (5 µg/mL) cultures and then sc

286 by PCR.

287 *Is 768: NA1000 me::me-apex2-flg-egfp Kan[®]

287 The RNE-Apex2FlgC-EGFPC-2 plasmid was recombined into the <i>me* locus
-

Js768: NA1000 rne::rne-apex2-flg-egfp Kan^R

- 287 <u>Js768: N</u>
288 The RNE
289 mating a
290 were firs
291 Result:
292 RNasel
293 APEX2 288
289
290
291
292
- 286 by PCR.

287 *Is 268: NA1000 me::rne-apex2-fig-egfp Kan[®]

288 The RNE-Apex2FigC-EGFPC-2 plasmid was recombined into the <i>me* locus in NA1000 via

289 mating and the selection was carried out on PYE + Kan plates. The 289 The RNE-Apex2FigC-EGFPC-2 plasmind was recombined into the rne locus in NA1000 via
289 The RNE-Apex2FigC-EGFPC-2 proximity tabeling was recombined into the rne locus in NA1000 via
289 Were first grown in PYE + Kan cult
-
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280 were first grown in PYE + Kan cultures and then screened by PCR.

291 Results:

292 RNaseE-APEX2 proximity labeling of RNA requires Alkyne-Phenol, H₂O₂, and

293 APEX2

293 APEX2 291 Results:
292 RNaseE-APEX2 proximity labeling of RNA requires Alkyne
293 APEX2 291 Results:
292 RNaseE-*|*
293 APEX2 292 RNaseE-APEX2 proximity labeling of RNA requires Alkyne-Phenol, H₂0₂, and APEX2

 293 APEX2

295 free radical on the oxygen of alkyne-phenol, and this highly reactive species can react with
296 molecules within a 10-20 nm radius 13,14 (Fig 1A). It was shown that APEX2 can label RNA is
297 eukaryotic cells, ma 296 molecules within a 10-20 nm radius $^{13.12}$ (Fig 1A). It was shown that APEX2 can label RNA in eukaryotic cells, making this a useful experimental system for identifying localized RNAs $^{15.15.16}$. In order to perfo molecules within a 10-20 nm radius ^{som} (Fig 1A). It was shown that APEX2 can label times and experimental system for identifying localized RNAs in such that is started that the perform RNA proximity labeling in bacteria, 298

^{10,15,15}, In order to perform RNA proximity labeling in bacteria, we first generated a gene

299 fusion between RNase E and APEX2. RNase E is known to phase-separate into bacterial

300 fusion between RNase E and AP $10,15,16$. In order to perform RNA proximity labeling in bacteria, we first generated a gene 299 1 Usion between RNase E and APEX2. RNase E is known to phase-separate into bacteria

299 1 usion between RNase E and APEX2. RNase E is known to phase-separate into bacteria

300 in bacteria, we examined the subcellula 299 films are the RNase E and External and found that it has similar many and the RNAse E-APEX2 fusions are tolerated in bacteria, we examined the subcellular localization, cell fitness, and mRNA decay active of RNase E-A 300 promoting the mRNA decay process¹⁷. To determine whether APEX2 fusions are tolerated
302 in bacteria, we examined the subcellular localization, cell fitness, and mRNA decay activ
303 of RNase E-APEX2 fusions in the promoting the mRNA decay process¹⁷. To determine whether APEX2 tusions are toterated
302 in bacteria, we examined the subcellular localization, cell fitness, and mRNA decay activit
301 of RNase E-APEX2 tusions in the bac 303 of RNase E-APEX2 tusions in the bacterium *Caulobacter crescentus* (Fig 1B). We observed
304 that fusing APEX2 tusions in the bacterium *Caulobacter crescentus* (Fig 1B). We observed
305 not alter its ability to phase 303 of The RNAse E-RPEX2 fusions was exercted to the backerium Caulobacter is ability to phase esparate into BR-bodies (Fig 1B). In addition, since RNase E's ability to degrade mRNAs is essential for cell growth ^{11,18}, 305 not alter its ability to phase separate into BR-bodies (Fig 1B). In addition, since RNase E's
306 ability to degrade mRNAs is essential for cell growth ^{11,18}, we also examined the cellular
307 fitness of the RNase E-306 ability to degrade mRNAs is essential for cell growth ^{11,18}, we also examined the cellular fitness of the RNase E-APEX2 fusion and found that both CFUs and colony size were
308 the RNase E-APEX2 fusion and found tha ability to degrade mRNAs is essential for cell growth ^{11,18}, we also examined the cellular
indistinguishable from wild-type (Fig 1B). Finally, we compared the mRNA decay astivitie
abilitinguishable from wild-type (Fig 1B 308 indistinguishable from wild-type (Fig 1B). Finally, we compared the mRNA decay act
309 the RNase E-APEX2 fusion and found that it has similar rates of mRNA decay as com
310 to wild-type (Fig 1B), while 5S rRNA remaine 309 the RNase E-APEX2 fusion and found that it has similar rates of mRNA decay as compared
310 to wild-type (Fig 1B), while 5S rRNA remained stable. Altogether, we could not detect any
311 measurable differences in RNase E 310 to wild-type (Fig 1B), while 5S rRNA remained stable. Altogether, we could not detect any
311 measurable differences in RNase E-function when fused to APEX2. To examine whether
312 the APEX2 fusion can be used to label 311 measurable differences in RNase Efunction when fused to APEX2. To examine whether
312 the APEX2 fusion can be used to label RNA we added all combinations of proximity labellin
313 reactants (H₂O₂ and Alkyne-Phenol 312 the APEX2 fusion can be used to label RNA we added all combinations of proximity label
313 reactants (H₂O₂ and Alkyne-Phenol) in combination with or without the RNase E-APEX2
314 fusion (Fig 1C). Each of the proxim 313 reactants (H₂O₂ and Alkyne-Phenol) in combination with or without the RNase E-APEX2
314 tusion (Fig 1C). Each of the proximity labeling conditions were performed, and then we
315 extracted the RNA and used copper 314 fusion (Fig1C). Each of the proximity labeling conditions were performed, and then we
315 extracted the RNA and used copperclick chemistry to conjugate a Cy5-azide to the RNA
316 The RNA was then deposited on a positi 315 extracted the RNA and used copper click chemistry to conjugate a Cy5-azide to the RNA
316 The RNA was then deposited on a positively charged nylon membrane using a dot blot
317 apparatus, and the Cy5 signal was measure 316 The RNA was then deposited on a positively charged nylon membrane using a dot blot
317 apparatus, and the Cy5 signal was measured in a fluorescent gelimager. We find that on
318 in the presence of APEX2, H₂O₂, and 317 apparatus, and the Cy5 signal was measured in a fluorescent gel imager. We find that
318 in the presence of APEX2, H₂O₂, and alkyne-phenol do we observe RNA labeling (Fig 1C
319 suggesting that APEX2 proximity lab in the presence of APEX2, H₂O₂, and alkyne-phenol do we observe RNA labeling (Fig 1C),
319 in the presence of APEX2, H₂O₂, and alkyne-phenol do we observe RNA labeling (Fig 1C),
319 suggesting that APEX2 proximity 319 suggesting that APEX2 proximity labeling reactions occurred. To determine whether the Cy5 signal was a result of RNA, or contaminating DNA we performed RNase A or DNase I digestions on our samples before spotting them 329 System at APEX produces a result of RNA, or contaminating DNA we performed RNase A or DNase dialy dialy dialy discussed at the action of the action of MNA, or contaminating DNA we performed RNase A or DNase I dialy an

²² Cysestions on our samples before spotting them on the dot blot (Fig 1C). Here we see that
322 RNase A treatment leads to a complete loss of Cy5-fluorescence, while DNase I treatment
323 RNase A treatment leads to a co 322 RNase A treatment leads to a complete loss of Cy5-fluorescence, while DNase Itreatment
323 led to no difference in fluorescence signal, suggesting that our Cy5 fluorescence is from
324 RNA.
325 Rapid labeling of cellul 323 Ied to no difference in fluorescence signal, suggesting that our Cy5 fluorescence is from
323 Rapid labeling of cellular RNA with Alkyne-Phenol
326 One of the major challenges of studying bacterial mRNA decay is the ve 324 RNA.
325 Rapid labeling of cellular RNA with Alkyne-Phenol
326 One of the major challenges of studying bacterial mRNA decay is the very short
327 lifetimes of cellular mRNAs. Most bacterial mRNAs in rapidly growing spe 325 Rapi
326 On
327 lifetin
328 lives I
329 To ad
330 used 325 Rapid tabeling of cellular HWA With Alkyne-Phenot
326 One of the major challenges of studying bacterial mRN
327 lifetimes of cellular mRNAs. Most bacterial mRNAs in rapi
328 Ives between 1-4 minutes ^{19,20}, making it 327 lifetimes of cellular mRNAs. Most bacterial mRNAs in rapidly growing species have
328 lives between 1-4 minutes ^{19,20}, making it hard to harvest the RNA before they are de
329 To address this technical limitation, w lives between 1-4 minutes 19,20, making it hard to harvest the RNA before they are degraded.
329 To address this technical limitation, we first optimized the Alkyne-phenol concentration
330 used for labeling cells and fou 330 used for labeling cells and found that 2.5 mM Alkyne-Phenol robustly labels cellular RNA
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Figure 1333 (FIG 2). Implies a time-course of Haustanian intervals of Haustanian intervalsed upon longer proximity labeling reaction times to 1 minute reaction times. We performed a time-course of Haos in a sub-minutes tim 333

333 found that robust labeling could be achieved in as little as 15 seconds, while labeling

334 increased upon longer proximity labeling reaction times (Fig 2). Importantly, the labeli

335 reaction occurs on the sub 334 increased upon longer proximity labeling reaction times (Fig 2). Importantly, the label
335 reaction occurs on the sub-minutes timescale, making this method well-suited to the
333 comper click-chemistry of Azide-Biotin streation occurs on the sub-minutes timescale, making this method well-suited to the use
an exponentially growing bacterial cells.
334 in exponentially growing bacterial cells.
339 APEX2 proximity labeling of RNA is rather 335 in exponentially growing bacterial cells.
337 Copper click-chemistry of Azide-Biotin and streptavidin purification of labeled
338 RNA.
339 APEX2 proximity labeling of RNA is rather flexible due to the diversity of azid Copper click-chemistry of Azide-B
338 RNA.
339 APEX2 proximity labeling of RNA is
230 commercially available for copper catal
341 RNA-sequencing, we altered the azide fr
342 streptavidin-mediated RNA purification
343 RNA i 338

2012 Copper clientiately of AZide-Biothi and streptavidin perimetation of labeled

338 APEX2 proximity labeling of RNA is rather flexible due to the diversity of azides

339 commercially available for copper catalyzed 339

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347 can be

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351 sample

352 isolate

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354 enr 339 Commercialty available for copper catalyzed click chemistry. To isolate cellular RNA
341 RNA-sequencing, we altered the azide from azide-Cy5 dye to azide-biotin to allow for
312 SNA is extracted from cells and conjugat 341 RNA-sequencing, we altered the azide from azide-Cy5 dye to azide-biotin to allow for
341 RNA-sequencing, we altered the azide from azide-Cy5 dye to azide-biotin to allow for
342 RNA is extracted from cells and conjugat 342 streptavidin-mediated RNA purification from the cell (Fig 3A). In this approach, labeled RNA is extracted from cells and conjugated to azide-biotin using copper catalyzed clic chemistry, which allows for the subsequen 343 RNA is extracted from cells and conjugated to azide-biotin using copper catalyzed click
244 chemistry, which allows for the subsequent purification of the labeled RNA using
345 streptavidin-resin. While copper can clea 344 chemistry, which allows for the subsequent purification of the labeled RNA using
345 streptavidin-resin. While copper can cleave RNA, using a short incubation time for the
346 click reaction minimizes RNA cleavage by 345 streptavidin-resin. While copper can cleave RNA, using a short incubation time for click reaction minimizes RNA cleavage by copper (Fig S2). After click chemistry, the can be purified under stringent conditions and el ²¹⁴ strept in the public strept and strept in the resin under strept in the resin of the resin under dentating
348 click reaction minimizes RNA cleavage by copper (Fig S2). After click chemistry, the RN
343 conditions. 347 can be purified under stringent conditions and eluted from the resin under denaturing
348 conditions. When applied to the RNase E-APEX2 fusion presented earlier, we found that
349 contrifugation (Fig 3)^{11,21}. In par 348 conditions. When applied to the RNase E-APEX2 fusion presented earlier, we found the eluted RNA profile indeed matched that of BR-body RNA isolated via density
350 centrifugation (Fig 3)^{11,21}. In particular, the rRN

248 the eluted RNA profile indeed matched that of BR-body RNA isolated via density
349 the eluted RNA profile indeed matched that of BR-body RNA isolated via density
351 contrifugation (Fig 3)^{11,21}. In particular, the rR 359 centrifugation (Fig 3)^{11,21}. In particular, the rRNA and tRNAs were depleted from t
351 solated BR-body RNA. Importantly though, while centrifugation-based isolation
352 isolated BR-body RNA. Importantly though, whil centrifugation (Fig 3)¹¹³². In particular, the rRNA and tRNAs were depleted from the RNA
352 isolated BR-body RNA. Importantly though, while centrifugation-based isolation of BR-
350 isolated BR-body RNA. Importantly tho 352 isolated BR-body RNA. Importantly though, while centrifugation-based isolation of
353 isolated BR-body RNA. Importantly though, while centrifugation-based isolation of
353 encicled RNAs, APEX2 proximity labeling requi oriched RNAs, APEX2 proximity labeling required only 4 mL of cell culture and 3 days of
355 hands-on work, making it higher throughput.
353 Boscussion:
357 RNP complexes are important regulators of RNA biology, including t 355 hands-on work, making it higher throughput.

356 Discussion:

357 RNP complexes are important regulators of RNA biology, including transcription, RNA

358 processing, transport, translation, and decay^{422, 24}. In addi 2356 Discussion:

8356 Discussion:

8357 RNP complexes are important regulators of F

9358 processing, transport, translation, and decay

8359 these complexes have been increasingly four

360 structures, which help to faci 357 RNP complexes
358 processing, train
359 these complexes
360 structures, whicrocomplexes
361 cycle via phase
362 increasingly im
363 have been deve
364 the procedures
365 labeling can rap
366 have known pat processing, transport, translation, and decay^{4,22-24}. In addition to RNP complex format
359 these complexes have been increasingly found to localize into biomolecular condenss
360 structures, which help to facilitate the processing, transport, translation, and decay ²²–2². In addition to RNP complex formation,
359 these complexes have been increasingly found to localize into biomolecular condensate
360 structures, which help to facilit 359 structures, which help to facilitate the spatial organization of the stages in the mRNA life cycle via phase-separation 11.17.25-27. As realizations that RNA localization has become increasingly important in bacteria, cycle via phase-separation 11.17.25-27. As realizations that RNA localization has become
362 increasingly important in bacteria, methods to identify the population of localized RNAs
363 have been developed, yet these metho cycle via phase-separation 11,17,25–27. As realizations that RNA localization has become
increasingly important in bacteria, methods to identify the population of localized RNA
have been developed, yet these methods have b 363 have been developed, yet these methods have been limited due to the long timescale of
364 the procedures compared to the short mRNA lifetimes in bacteria. APEX2 proximity
365 labeling can rapidly label RNA and can be e 364 the procedures compared to the short mRNA lifetimes in bacteria. APEX2 proximity
365 labeling can rapidly label RNA and can be easily genetically fused to genes whose proteir
366 have known patterns of subcellular loca 365 the procedure procedure of subcellar and can be easily genetically fused to genes whose procedures compare
366 have known patterns of subcellular localization. APEX2 proximity labeling of RNA required to the short matr 366 have known patterns of subcellular localization. APEX2 proximity labeling of RNA requires
15 35 66 have known patterns of subcellular localization. Application, 35

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sufficient RNA for downstream analysis. The reactivity radius of APEX2 is estimated to be 10-20nm²⁸, suggesting it gives high spatial precision. Genetic fusion of APEX2 is estimated to be dependent and APEX2 is similar i 368 10-20nm²⁸, suggesting it gives high spatial precision. Genetic fusion of APEX2 is easy to
368 10-20nm²⁸, suggesting it gives high spatial precision. Genetic fusion of APEX2 is easy to
371 tunctional impacts when fu 10-20nm²², suggesting it gives high spatial precision. Genetic fusion of APEX2 is easy to

10-20nm²², similar in size to a fluorescent protein and has no observable

1371 (unctional impacts when fused to RNase E, so we 371

371 functional impacts when fused to RNase E, so we anticipate that this is unlikely to dis

372 target protein function. Altogether, APEX2 proximity labeling of RNA will help to acce

373 to a fluorency of localized 272 target protein function. Altogether, APEX2 proximity labeling of RNA will help to accelerate

373 the discovery of localized RNAs in bacteria.

374 Acknowledgements:

375 NH grant R35GM124733 to JMS. WSU Career Chair A 373 the discovery of localized RNAs in bacteria.
374 Acknowledgements:
375 NIH grant R35GM124733 to JMS. WSU Career Chair Award to JMS. NIH T32GM142519-03
376 to HY.
377 Figure 1. APEX2 proximity labeling of RNA in bacteri 374 Acknowledgements:
375 NIH grant R35GM124733 to JMS. WSU Care
376 to HY.
377 Figure legends:
379 Figure 1. APEX2 proximity labeling of RNA
380 A) Schematic of APEX2 labeling of RNA prote
381 the major protein that scaff 374 Acknowledgements:
375 NIH grant R35GM124733 t
376 to HY.
377 Figure legends:
379 Figure legends:
380 A) Schematic of APEX2 lab
381 the major protein that scafe
382 alkyne-phenol, and labele
383 extracted from the cells

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376 to HY.

377 Figure legends:

379 Figure legends:

379 Figure 1. APEX2 proximity tabeling of RNA in bacterial cells with minimal perturbation

4) Schematic of APEX2 labeling of RNA protocol. The APEX2 protein was f 377

378 Figure

380 A) Sch

381 the ma

382 alkyne

382 alkyne

383 extract

384 click c

385 (growtl

385 shows

385 shows

387 Growtl

387 Growtl

389 half-lif

390 APEX2

391 were p
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- 377 379 Figure 1. APEX2 pro
380 A) Schematic of API
381 the major protein th
382 alkyne-phenol, and
383 extracted from the c
384 click chemistry. B) /
385 (growth rate). Left:
386 shows that fusion d
387 Growth of RNase E-
388
- **389 All the measure 1. All the minimal periodic state in the minimal periodic The APEX2 protein was fused to RNase E, the major protein that scaffolds BR-bodies. Cells are incubated in media containing alkyne-phenol, a**
-
- 1081 Startingtheory of APEX2 and Galear SBR-bodies. Cells are incubated in media containing

382 alkyne-phenol, and labeled was initiated with H₂O₂. After a brief labeling reaction, RNA was

383 extracted from the cell 382 alkyne-phenol, and labeled was initiated with H₂O₂. After a brief labeling reaction, RN
383 extracted from the cells, and Cy5-azide was conjugated to the RNA by copper cataly
384 click chemistry. B) APEX2 fusion d 383 actracted from the cells, and Cy5-azide was conjugated to the RNA by copper catalyzed
384 click chemistry. B) APEX2 fusion does not dramatically impact localization or function
385 (growth rate). Left: *In vivo* locali 384 cick chemistry. B) APEX2 tusion does not dramatically impact localization or function
385 (growth rate). Left: *In vivo* localization of RNase E-msfGFP vs RNase EAPEX2-msfGFP
386 shows that fusion does not impact the f 383 (growth rate). Left: *In vivo* localization of RNase E-msfGFP vs RNase EAPEX2-msfGFP
386 (growth rate). Left: *In vivo* localization of RNase E-msfGFP vs RNase EAPEX2-msfGFP
386 click chemistry. B) Applements by qRT-PC Solution does not impact the formation of BR-bodies. Scale bar is 1 pm. Middles shows that fusion does not impact the formation of BR-bodies. Scale bar is 1 pm. Middles for with of RNase E-APEX2 fusion is similar to wild t 387 Growth of RNase E-APEX2 fusion is similar to wild type *Caulobacter* cells. Right: mRNA
388 half-life measurements by qRT-PCR show that RNase E-APEX2 degrades mRNAs with a
389 similar half-life to wild-type. Data are 388 and F-life measurements by qRT-PCR show that RNase E-APEX2 degrades mRNAs with a similar half-life to wild-type. Data are from three biological and technical replicates. C) APEX2 labeling requires H₂O₂ and alkyne-388 similar half-life to wild-type. Data are from three biological and technical replicates. C)
380 similar half-life to wild-type. Data are from three biological and technical replicates. C)
390 APEX2 labeling requires APEX2 labeling requires H₂O₂ and alkyne-phenol to label RNAs. RNA labeling reactions
391 APEX2 labeling requires H₂O₂ and alkyne-phenol to label RNAs. RNA labeling reactions
392 for Cy5 fluorescence in a gel imager 391 Were placed on a nylon membrane to bind to the RNA in a dot blot apparatus and scann
392 for Cy5 fluorescence in a gel imager. As a control, the RNA samples were incubated for
393 for Cy5 fluorescence in a gel imager. 392 for Cy5 fluorescence in a gel imager. As a control, the RNA samples were incubated for 2
393 for Cy5 fluorescence in a gel imager. As a control, the RNA samples were incubated for 2
393 horts with DNase I and RNase A. 393 for Cystal and Rhase A. The RNA was then precipitated before being subjected the azide-Cy5 click chemistry reaction and was re-precipitated before being subjected the azide-Cy5 click chemistry reaction and was re-preci the azide-Cy5 click chemistry reaction and was re-precipitated before being filtered on the
aylon membrane in the dot blot apparatus.
396 Figure 2. APEX2 proximity labeling of RNA works rapidly. Optimization of APEX2 label 395 nylon membrane in the dot blot apparatus.
396 Figure 2. APEX2 proximity labeling of RNA works rapidly. Optimization of APEX2 labeling
397 of RNA with Alkyne-Phenol. Top: Alkyne-phenol titration reveals that peak labeli Eigure 2. APEX2 proximity labeling of RNA
397 of RNA with Alkyne-Phenol. Top: Alkyne-ph
398 with 2.5 mM Alkyne-Phenol. RNA was labele
intensity was measured in a gel imager. Bot
400 labeled in the scheme shown in Fig 1A, a 397 of RNA with Alkyne-Phenol. Top: Alkyne-phenol titration reveals that peak labeling occurs
398 with 2.5 mM Alkyne-Phenol. RNA was labeled in the scheme shown in Fig 1A, and the Cy5
399 intensity was measured in a gel im 398 with 2.5 mM Alkyne-Phenol. RNA was labeled in the scheme shown in Fig 1A, and the Cy5
399 intensity was measured in a gel imager. Bottom: Time course of APEX2 labeling. RNA was
400 labeled in the scheme shown in Fig 1A 399 intensity was measured in a gel imager. Bottom: Time course of APEX2 labeling. RNA was labeled in the scheme shown in Fig 1A, and RNA labeling is apparent in as short as 15
16
- 399 in a general control of APEX2 in a general control of APEX2 labeled in the scheme shown in Fig 1A, and RNA labeling is apparent in as short as 15
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- H_2O_2 incubation,
403 Figure 3. APEX2 proximity labeled RNA can be isolated by streptavidin purification.
403 Figure 3. APEX2 proximity labeled RNA can be isolated by streptavidin purification.
404 Schematic of the con 403 Figure 3. APEX2 |
404 Schematic of the
405 streptavidin purif
406 RNA profiles of th
407 The bottom two b
408 differential centri
409 Figure S1. RNase
410 *Hfq-FLAG (LS437*
411 transferred to PV
412 Figure S2. Effect
4 Figure 3. AT EXZ proximity also the APEX2 fusion. The MERCAL AND Restricted RNA contrastion of the conjugation of biotin-azide to clicked alkyne-phenol and the resulting
404 Schematic of the conjugation of biotin-azide to 404 Steptavidin purification. B) Streptavidin purification of biotinylated RNAs. Tapestation
406 RNA profiles of the lysates and elution fractions of biotinylated proximity labeled RNAs.
407 The bottom two bands are tRNAs,
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-
-

- 406 RNA profiles of the lysates and elution fractions of biotinylated proximity labeled RNAs.

407 The bottom two bands are tRNAs, which are known to be highly depleted in BR-bodies fr

408 differential centrifugation-base 407 The bottom two bands are tRNAs, which are known to be highly depleted in BR-bodies fr
408 differential centrifugation-based isolation of BR-bodies^{11,21}.
409 Figure S1. RNase E-APEX2-FLAG Western Blot. Protein lysates 408

408 differential centrifugation-based isolation of BR-bodies ^{11,2}.

409 Figure S1. RNase E-APEX2-FLAG Western Blot. Protein lysates generated from NA1000,

410 Hfg-FLAG (LS4379) and RNase E-APEX2-FLAG (JS767) were r differential centrifugation-based isolation of BR-bodies³¹¹¹².

409 **Figure S1. RNase E-APEX2-FLAG Western Blot**. Protein lys

410 *Hfq-FLAG (LS4379)* and *RNase E-APEX2-FLAG (JS767)* were

411 transferred to PVDF, and p Figure S1. Reasons and RNase E-APEX2-FLAG (JS767) were run on an SDS PAGE gel,

410 Figure S2. Effects of copper catalyzed click reaction on RNA integrity. RNA extracted

411 transferred to PVDF, and probed with an anti-FL 411 Hamstered to PVDF, and probed with an anti-FLAG antibody.
411 Intensite to PVDF, and probed with an anti-FLAG antibody.
412 Figure S2. Effects of copper catalyzed click reaction on RNA integrity. RNA extraction
413 fro Figure S2. Effects of copper catalyzed click reaction on RN

413 from APEX2 proximity labeling reactions were ran on an Agiler

414 harboring RNase E-APEX2 fusion. The APEX2 labeled RNA wa

5 Sulfate under similar conditio 412 Figure 32. Enstate of copper catalyzed click reaction on an Agilent Tapestation from cells
413 from APEX2 proximity labeling reactions were ran on an Agilent Tapestation from cells
414 harboring RNase E-APEX2 fusion. T 414 harboring RNase E-APEX2 fusion. The APEX2 labeled RNA was also incubated with Co
415 Sulfate under similar conditions for click chemistry reactions where we observe minim
416 RNA degradation from the copper.
413 Refere 415 Sulfate under similar conditions for click chemistry reactions where we observe minimal
416 RNA degradation from the copper.
417 11 Aannaiah, S., Livny, J., and Amster-Choder, O. (2019). Spatiotemporal Organization of
 416 RNA degradation from the copper.

417

418 References:

419 1. Kannaiah, S., Livny, J., and Amster-Choder, O. (2019). Spatiotemporal Organization of

420 the E. coli Transcriptome: Translation Independence and Engageme 417

417

418 References:

419 1. Kannaiah, S., Livny, J., and Ams

420 the E. coli Transcriptome: Trans

421 Mol. Cell 76, 574-589.e7. https

422 2. Irastortza-Olaziregi, M., and Am

423 prokaryotes: Where, when, hov

424 4189014222348242424243243343344332 418 References:

419 1. Kannaiah, S

420 the E. coli Tr

421 Mol. Cell 76

423 prokaryotes

424 https://doi.c

425 3. Campos, M.

426 genetic infolial

427 https://doi.c

428 4. Nandana, V.

429 bacterial RN

430 https://d
- 1. Attns://doi.org/10.1015/j.mib.2013.01.007.
421 the E. coli Transcriptome: Translation Independence and Engagement in Regulation.
421 Mol. Cell 76, 574-589.e7. https://doi.org/10.1016/j.molcel.2019.08.013.
422 2. Irastor Mol. Cell 76, 574-589.e7. https://doi.org/10.1016/j.molcel.2019.08.013.

422 2. Irastortza-Olaziregi, M., and Amster-Choder, O. (2021). RNA localization in

423 prokaryotes: Where, when, how, and why. Wiley Interdiscip. Re 421 Mol. Celt 70, 574-589.7. https://doi.org/10.1018/j.mol. Celt 70.12012. RNA localization
422 2. Irastortza-Olaziregi, M., and Amster-Choder, O. (2021). RNA localization
424 https://doi.org/10.1002/wrna.1615.
425 3. Camp
-
- 123 prokaryotes: Where, when, how, and why. Wiley Interdiscip. Rev. RNA 12, e

122 thttps://doi.org/10.1002/wma.1615.

125 3. Campos, M., and Jacobs-Wagner, C. (2013). Cellular organization of the tra

126 genetic informat 424 prokaryotes: When, How, and Wiley Interdiscipe. The times: Michael at the second of the transfer of genetic information. Curr. Opin. Microbiol. 16, 171–176.
425 3. Campos, M., and Jacobs-Wagner, C. (2013). Cellular org 425 3. Campos, M., and Jacobs-Wagner, C
426 genetic information. Curr. Opin. Mic
6427 https://doi.org/10.1016/j.mib.2013.4
428 4. Nandana, V., and Schrader, J.M. (20)
6430 https://doi.org/10.1016/j.mib.2021.4
431 5. Dugar, genetic information. Curr. Opin. Microbiol. 16, 171–176.

Attps://doi.org/10.1016/j.mib.2013.01.007.

428 4. Nandana, V., and Schrader, J.M. (2021). Roles of liquid-liquid phase separation in

429 bacterial RNA metabolism. 427 genetic information. Curr. Opin. Hicrobiol. 16, 171–176.
427 https://doi.org/10.1016/j.mib.2013.01.007.
428 4. Nandana, V., and Schrader, J.M. (2021). Roles of liquid-li
62021. Microbiol. 61, 91-
430 https://doi.org/10 428 4. Nandana, V., and Schrader, J.M. (2021). Rolaton:
429 bacterial RNA metabolism. Curr. Opin. Micr
1430 https://doi.org/10.1016/j.mib.2021.03.005.
431 5. Dugar, G., Svensson, S.L., Bischler, T., Wäld
1432 Sharma, C.M.
- 429 bacterial RNA metabolism. Curr. Opin. Microbiol. 61, 91–98.
430 https://doi.org/10.1016/j.mib.2021.03.005.
431 5. Dugar, G., Svensson, S.L., Bischler, T., Wäldchen, S., Reinhardt, R., Sauer, M., and
432 Sharma, C.M. (2 429 bacterial Riva metabolism. Curr. Opin. Microbiol. 61, 91–98.
430 https://doi.org/10.1016/j.mib.2021.03.005.
431 5. Dugar, G., Svensson, S.L., Bischler, T., Wäldchen, S., Reinhal
432 Sharma, C.M. (2016). The CsrA-FliW n 431 5. Dugar, G., Svensson, S.L., Bischler, T., Wäld
432 Sharma, C.M. (2016). The CsrA-FliW networ
433 function flagellin mRNA in Campylobacter je
https://doi.org/10.1038/ncomms11667.
17 432 Sharma, C.M. (2016). The CsrA-FliW network controls polar localization of the dual
433 Sharma, C.M. (2016). The CsrA-FliW network controls polar localization of the dual
434 function flagellin mRNA in Campylobacter jej
- 433 function flagellin mRNA in Campylobacter jejuni. Nat. Commun. 7, 11667.
434 https://doi.org/10.1038/ncomms11667.
17 434 https://doi.org/10.1038/ncomms11667.
-
-
-
-
- (2024). An experimental framework to assess biomolecular condensates in bacteria.

437 (2024). An experimental framework to assess biomolecular condensates in bacteria.

437 Mat. Commun. 15, 3222. https://doi.org/10.1038/s Mat. Commun. 15, 3222. https://doi.org/10.1038/s41467-024-47330-4.

438 7. Darnell, R.B. (2010). HITS-CLIP: panoramic views of protein-RNA regulation in living

cells. Wiley Interdiscip. Rev. RNA 1, 266-286. https://doi.or 1437 Communities, 15, 0222. https://doi.org/10.1038/s41467 (1912). HTS-CLIP: panoramic views of protein-RNA regular cells. Wiley Interdiscip. Rev. RNA 1, 266-286. https://doi.org/10.1002/w

440 8. Hör, J., Di Giorgio, S.,
-
-
- cells. Wiley Interdiscip. Rev. RNA 1, 266–286. https://doi.org/10.1002/wrna.31.

440 8. Hör, J., Di Giorgio, S., Gerovac, M., Venturini, E., Förstner, K.U., and Vogel, J. (2020).

441 Grad-seq shines light on unrecognized ettes. Wiley Interdiscip. Rev. Rev. Rev. Rep. 200. Rep. 200. Rep. 200. Rev. Rev. Rev. 200. Rev. Rev. 200. Rev. 200. Bettes in the mode by the determine lasher light on unrecognized RNA and protein complexes in the mode htt Crad-seq shines light on unrecognized RNA and protein complexes in the model

442 bacterium Escherichia coli. Nucleic Acids Res. 48, 9301–9319.

443 https://doi.org/10.1093/nar/gkaa676.

444 9. Li, Y., Tian, C., Liu, K., Z bacterium Escherichia coli. Nucleic Acids Res. 48, 9301–9319.

443 https://doi.org/10.1093/nar/gkaa676.

444 9. Li, Y., Tian, C., Liu, K., Zhou, Y., Yang, J., and Zou, P. (2020). A Clickable APEX Pro

445 Proximity-Depende 442 bacterium Escherichia coli. Tuctoric Acids Res. 49, 3001–9319.

443 bttps://doi.org/10.1093/nar/gkaa676.

444 9. Li, Y., Tian, C., Liu, K., Zhou, Y., Yang, J., and Zou, P. (2020). A Cl

445 Proximity-Dependent Proteomi 1444 9. Li, Y., Tian, C., Liu, K., Zhou, Y., Yang, J

Proximity-Dependent Proteomic Profil

1445 Proximity-Dependent Proteomic Profil

1446 Https://doi.org/10.1016/j.chembiol.20

10. Padron, A., Iwasaki, S., and Ingolia, N Froximity-Dependent Proteomic Profiling in Yeast. Cell Chem. Biol. 27, 858-865.e8.

Attps://doi.org/10.1016/j.chembiol.2020.05.006.

447 10. Padron, A., Iwasaki, S., and Ingolia, N.T. (2019). Proximity RNA Labeling by APEX 11. At-Husini, Public Proteomic Proteins, N.T. (2019). Proximity RNA Labeling by APEX-Sections, N.T. (2019). Proximity RNA Labeling by APEX-Sections, A., Wasaki, S., and Ingolia, N.T. (2019). Proximity RNA Labeling by APEX 10. Padron, A., Iwasaki, S., and Ingolia, N.T. (2019). P.

Reveals the Organization of Translation Initiation

448 Reveals the Organization of Translation Initiation

449 Granules. Mol Cell 75, 875-887 e5. https://doi.org
 Reveals the Organization of Translation linitiation Complexes and Repressive RNA

449 Granules. Mol Cell 75, 875-887 e5. https://doi.org/10.1016/j.molcel.2019.07.030.

450 11. Al-Husini, N., Tomares, D.T., Pfaffenberger, Z Granules. Mol. Cell 75, 875-887 e5. https://doi.org/10.1016/j.molcel.2019.07.030.
450 I1. Al-Husini, N., Tomares, D.T., Pfaffenberger, Z.J., Muthunayake, N.S., Samad, M.A.
451 T., Bitar, O., Aretakis, J.R., Bharmal, M.-H.M 449 Franchines. Mol Cell 75, 875-887. Https://doi.org/10.1016/j.molece.2019.07.030.

11. Al-Husini, N., Tomares, D.T., Pfaffenberger, Z.J., Muthunayake, N.S., Samad, M.A.,

11. Bitar, O., Aretakis, J.R., Bharmal, M.-H.M., 151 1., Bitar, O., Aretakis, J.R., Bharmal, M.-H.M., Gega, A., et al. (2020). BR-bodies provide

1452 selectively permeable condensates that stimulate mRNA decay and prevent release of

1453 decay intermediates. Mol. Cell
-
-
- selectively permeable condensates that stimulate mRNA decay and prevent release of
decay intermediates. Mol. Cell 78, 670-682.e8.
https://doi.org/10.1016/j.molcel.2020.04.001.
454
12. Padrón, A., Iwasaki, S., and Ingola., 453 decay intermediates. Mol. Cell 78, 670-682.e8.

454 https://doi.org/10.1016/j.molcel.2020.04.001.

455 12. Padrón, A., Iwasaki, S., and Ingolia, N.T. (2019). Proximity RNA Labelling by APEX-Seq

Reveals the Organizatio 454 decay micharcalics. Hol. Oct 70, 070 0021.00.

454 https://doi.org/10.1016/j.molcel.2020.04.001.

455 12. Padrón, A., Iwasaki, S., and Ingolia, N.T. (2019).

456 Reveals the Organization of Translation Initiatio

457 G 12. Padrón, A., Iwasaki, S., and Ingolia, N.T. (2019)

1456 Reveals the Organization of Translation Initiatio

1457 Granules. Mol. Cell 75, 875-887.e5. https://doi

13. Kalocsay, M. (2019). APEX Peroxidase-Catalyze

14. Jä Reveals the Organization of Translation Initiation Complexes and Repressive RNA

457 Granules. Mol. Cell 75, 875-887.e5. https://doi.org/10.1016/j.molcel.2019.07.030.

458 13. Kalocsay, M. (2019). APEX Peroxidase-Catalyzed Granules. Mol. Cell 75, 875-887.e5. https://doi.org/10.1016/j.molcel.2019.07.030
458 13. Kalocsay, M. (2019). APEX Peroxidase-Catalyzed Proximity Labeling and Multiplex
459 Quantitative Proteomics. In Proximity Labeling: M 458 13. Kalocsay, M. (2019), APEX Peroxidase-Catalyzed Proximity Labeling and Multiplexe

458 Quantitative Proteomics. In Proximity Labeling: Methods and Protocols, M. Sunbul

460 A. Jäschke, eds. (Springer), pp. 41–55. ht
- Quantitative Proteomics. In Proximity Labeling: Methods and Protocols, M. Sunbul and A. Jäschke, eds. (Springer), pp. 41–55. https://doi.org/10.1007/978-1-4939-9537-0_4
461 14. Hung, V., Udeshi, N.D., Lam, S.S., Loh, K.H., A. Jäschke, eds. (Springer), pp. 41–55. https://doi.org/10.1007/978-1-4939-9537-0_4.

461 14. Hung, V., Udeshi, N.D., Lam, S.S., Loh, K.H., Cox, K.J., Pedram, K., Carr, S.A., and Ting,

462 A.Y. (2016). Spatially resolved 461 14. Hung, V., Udeshi, N.D., Lam, S.S., Loh, K.H., Cox, K.J., Pedram, K., Carr, S.A., and Ting A.Y. (2016). Spatially resolved proteomic mapping in living cells with the engineered
463 A.Y. (2016). Spatially resolved pr
- 462 A.Y. (2016). Spatially resolved proteomic mapping in living cells with the engineered

463 A.Y. (2016). Spatially resolved proteomic mapping in living cells with the engineered

463 Proximity Labeling: A. Reinold, L., 463 Peroxidase APEX2. Nat. Protoc. 11, 456–475. https://doi.org/10.1038/nprot.2016.014
464 15. Weissinger, R., Heinold, L., Akram, S., Jansen, R.-P., and Hermesh, O. (2021). RNA
465 Proximity Labeling: A New Detection Tool 464 15. Weissinger, R., Heinold, L., Akram, S., Jansen, R.-P., and Hermesh, O. (2021). RNA

465 Proximity Labeling: A New Detection Tool for RNA–Protein Interactions. Molecules 26,

466 2270. https://doi.org/10.3390/molecu
- Proximity Labeling: A New Detection Tool for RNA–Protein Interactions. Molecules.

466 2270. https://doi.org/10.3390/molecules26082270.

467 16. Padrón, A., and Ingolia, N. (2022). Analyzing the Composition and Organizatio 466 Proximity Labeling: A New Detection Tool for RNA–Protein Interactions. Molecules 26,
466 2270. https://doi.org/10.3390/molecules26082270.
467 16. Padrón, A., and Ingolia, N. (2022). Analyzing the Composition and Organi 16. Padrón, A., and Ingolia, N. (2022). Analyzing the Com

Ribonucleoprotein Complexes by APEX-Seq. Method

289. https://doi.org/10.1007/978-1-0716-1975-9_17

18 468 Ribonucleoprotein Complexes by APEX-Seq. Methods Mol. Biol. Clifton NJ 2428, 2
289. https://doi.org/10.1007/978-1-0716-1975-9_17.
18 469 Ribonucleoprotein Complexes by APEX-Seq. Methods Mol. Biol. Clifton NJ 2428, 277–289.
289. https://doi.org/10.1007/978-1-0716-1975-9_17.
18 $\frac{1}{18}$ 299. https://doi.org/10.1007/978-10.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/978-11.1007/9

470 17. Al-Husini, N., Tomares, D.T., Bitar, O., Childers, W.S., and Schrader, J.M. (2018), alpha-

-
-

Bodies. Mol Cell 71, 1027-1039 e14. https://doi.org/10.1016/j.molcel.2018.08.003.

473 B. Christen, B., Abeliuk, E., Collier, J.M., Kalogeraki, V.S., Passarelli, B., Coller, J.A., Fer

474 M.J., McAdams, H.H., and Shapiro, 272 Bodies. Hot Get 77, 1027-1039 e14. https://doi.org/10.1016/j.includi.Levit.Col.col.col.

473 B. Christen, B., Abeliuk, E., Collier, J.M., Kalogeraki, V.S., Passarelli, B., Coller, J.A., Fe

474 B., McAdams, H.H., and S M.J., McAdams, H.H., and Shapiro, L. (2011). The essential genome of a bacterium. Mo
Syst Biol 7, 528-528. https://doi.org/10.1038/msb.2011.58.
476 19. Laalami, S., Zig, L., and Putzer, H. (2013). Initiation of mRNA decay Syst Biol 7, 528–528. https://doi.org/10.1038/msb.2011.58.

476 Syst Biol 7, 528–528. https://doi.org/10.1038/msb.2011.58.

476 19. Laalami, S., Zig, L., and Putzer, H. (2013). Initiation of mRNA decay in bacteria. Cell.
 475 Syst Biol 7, 528–528. https://doi.org/10.1038/msb.2011.58. Mol. Life Sci. CMLS 71, 1799–1828. https://doi.org/10.1007/s00018-013-1472-4.

478 20. Jenniches, L., Michaux, C., Popella, L., Reichardt, S., Vogel, J., Westermann, A.J., an

479 Barquist, L. (2024). Improved RNA stabilit 477 20. Jenniches, L., Michaux, C., Popella, L., Reichardt, S., Vogel, J., Westermann, A.J.,
Barquist, L. (2024). Improved RNA stability estimation through Bayesian modeling
reveals most Salmonella transcripts have subminu Barquist, L. (2024). Improved RNA stability estimation through Bayesian modeling

reveals most Salmonella transcripts have subminute half-lives. Proc. Natl. Acad. Sci.

U. S. A. 121, e2308814121. https://doi.org/10.1073/pn

- Francella Stability and Stability in the stability of baractic stability in the stability of the stability to U.S. A. (21, e2308814121. Https://doi.org/10.1073/pnas.2308814121.

482 Barticle Bacterial Ribonucleoprotein Bod U. S. A. 121, e2308814121. https://doi.org/10.1073/pnas.2308814121.
482 21. Muthunayake, N.S., Al-Husini, N., and Schrader, J.M. (2020). Differential Centrifugatio
483 to Enrich Bacterial Ribonucleoprotein Bodies (BR bodie 482 21. Muthunayake, N.S., Al-Husini, N., and Schrader, J.M. (2020). Differentia
483 to Enrich Bacterial Ribonucleoprotein Bodies (BR bodies) from Cauloba
484 crescentus. STAR Protoc. 1, 100205. https://doi.org/10.1016/j.x
- 485 22. Waters, L.S., and Storz, G. (2009). Regulatory RNAs in bacteria. Cell 136, 615–628.
486 https://doi.org/10.1016/j.cell.2009.01.043.
- to Enrich Bacterial Ribonucleoprotein Bodies (BR bodies) from Caulobacter

crescentus. STAR Protoc. 1, 100205. https://doi.org/10.1016/j.xpro.2020.100205.

485 22. Waters, L.S., and Storz, G. (2009). Regulatory RNAs in bac 484 crescentus. STAR Protoc. 1, 100205. https://doi.org/10.1016/j.xpro.2020.10
485 22. Waters, L.S., and Storz, G. (2009). Regulatory RNAs in bacteria. Cell 136, 615
486 https://doi.org/10.1016/j.cell.2009.01.043.
487 23.
-
-
-
-
-
-
- 484

485 crescentus. STAR Protoc. 1, 100203. https://doi.org/10.1016/j.cell.2009.01.043.

486 tttps://doi.org/10.1016/j.cell.2009.01.043.

487 23. Olejniczak, M., Jiang, X., Basczok, M.M., and Storz, G. (2022). KH domain p 22. Waters, L.S., and Victoria, C. (2009.), R. R. And Storz, G. (2002). KH domain proteins:
486 23. Olejniczak, M., Jiang, X., Basczok, M.M., and Storz, G. (2022). KH domain proteins:
488 Another family of bacterial RNA ma 23. Olejniczak, M., Jiang, X., Basczok, M.M., and

488 Another family of bacterial RNA matchmake

https://doi.org/10.1111/mmi.14842.

490 24. Holmqvist, E., and Vogel, J. (2018). RNA-bin

Microbiol. 16, 601–615. https://do Aas

Another family of bacterial RNA matchmakers? Mol. Microbiol. 117, 10–19.

https://doi.org/10.1111/mmi.14842.

490 24. Holmqvist, E., and Vogel, J. (2018). RNA-binding proteins in bacteria. Nat. Rev.

Microbiol. 16, 60 488 https://doi.org/10.1111/mmi.14842.
488 https://doi.org/10.1111/mmi.14842.
490 24. Holmqvist, E., and Vogel, J. (2018). RNA-binding proteins in bacteria. Nat. R
491 Microbiol. 16, 601–615. https://doi.org/10.1038/s41579 24. Holmqvist, E., and Vogel, J. (2018). R
491 Microbiol. 16, 601–615. https://doi.or
492 25. Nandana, V., Rathnayaka-Mudiyanse
493 Mousseau, C.B., Ortiz-Rodríguez, L.A
494 Mallikaarachchi, K.S., et al. (2023). T
495 of pr Microbiol. 76, 601–615. https://doi.org/10.1038/s41579-018-0049-5.
492 25. Nandana, V., Rathnayaka-Mudiyanselage, I.W., Muthunayake, N.S., Hatami, A.
493 Mousseau, C.B., Ortiz-Rodríguez, L.A., Vaishnav, J., Collins, M., Ge 491 Microbiol. 16, 601–615. https://doi.org/10.1053141579-016-6045-1
492 25. Nandana, V., Rathnayaka-Mudiyanselage, I.W., Muthunayake, N.S., Housseau, C.B., Ortiz-Rodríguez, L.A., Vaishnav, J., Collins, M., Gega
494 Mallik Mousseau, C.B., Ortiz-Rodríguez, L.A., Vaishnay, J., Collins, M., Gega, A.,

494 Mallikaarachchi, K.S., et al. (2023). The BR-body proteome contains a complex not protein-protein and protein-RNA interactions. Cell Rep. 42, Mallikaarachchi, K.S., et al. (2023). The BR-body proteome contains a compression of protein-protein and protein-RNA interactions. Cell Rep. 42, 113229.
495 of protein-protein and protein-RNA interactions. Cell Rep. 42, 11 495 of protein-protein and protein-RNA interactions. Cell Rep. 42, 113229.
496 of protein-protein and protein-RNA interactions. Cell Rep. 42, 113229.
497 26. Ladouceur, A.-M., Parmar, B.S., Biedzinski, S., Wall, J., Tope, 496 of protein-mail protein-mail protein-mail controls. Cell Apple 22, 113229.

496 https://doi.org/10.1016/j.celrep.2023.113229.

497 26. Ladouceur, A.-M., Parmar, B.S., Biedzinski, S., Wall, J., Tope, S.G., Coh

500 soub 26. Ladouceur, A.-M., Parmar, B.S., Biedzinski, S., Soubry, N., Reyes-Lamothe, R., and Weber, S.C.
499 bolymerase are biomolecular condensates tha
separation. Proc. Natl. Acad. Sci. 117, 18540–1
https://doi.org/10.1073/pna Soubry, N., Reyes-Lamothe, R., and Weber, S.C. (2020). Clusters of bacterial RNA

499 Soubry, N., Reyes-Lamothe, R., and Weber, S.C. (2020). Clusters of bacterial RNA

499 polymerase are biomolecular condensates that assem
-
- 499 polymerase are biomolecular condensates that assemble through liquid-liquid ph
499 separation. Proc. Natl. Acad. Sci. 117, 18540–18549.
601 https://doi.org/10.1073/pnas.2005019117.
602 27. Hondele, M., Sachdev, R., Hei 499 bolymeration. Proc. Natl. Acad. Sci. 117, 18540–18549.

501 https://doi.org/10.1073/pnas.2005019117.

502 27. Hondele, M., Sachdev, R., Heinrich, S., Wang, J., Vallotton, P., Fontoura, B.M.A., and

49 Weis, K. (2019). 500 separation. Froc. Natl. Acad. Sci. 117, 18540–18545.
501 https://doi.org/10.1073/pnas.2005019117.
502 27. Hondele, M., Sachdev, R., Heinrich, S., Wang, J., Vall.
Weis, K. (2019). DEAD-box ATPases are global regulat
504 502 27. Hondele, M., Sachdev, R., Heinrich, S., War
503 Weis, K. (2019). DEAD-box ATPases are glot
6504 organelles. Nature 573, 144–148. https://do 503 Weis, K. (2019). DEAD-box ATPases are global regulators of phase-separated
504 organelles. Nature 573, 144–148. https://doi.org/10.1038/s41586-019-1502-y.
504 19 504 organelles. Nature 573, 144–148. https://doi.org/10.1038/s41586-019-1502-y
19 504 organelles. Nature 570, 144–148. https://doi.org/10.10307341586-019-1502-y.

-
- 506 Ellisman, M.H., and Ting, A.Y. (2012). Engineered ascorbate peroxidase as a generated reporter for electron microscopy. Nat Biotechnol. 30, 1143-1148.
508 https://doi.org/10.1038/nbt.2375.
- 507 Electron electron microscopy. Nat Biotechnol. 30, 1143-1148.
508 Encoded reporter for electron microscopy. Nat Biotechnol. 30, 1143-1148.
https://doi.org/10.1038/hbt.2375. 508 https://doi.org/10.1038/nbt.2375.
- 508 https://doi.org/10.1038/nbt.2375.

B. APEX2 doesn't impact localization or protein function

Mock +RNase A +DNase I

B.

Streptavidin puri�ication

