

No.	K24-1118	
Project Title	Structures of the Gcn2 kinase on the ribosome	
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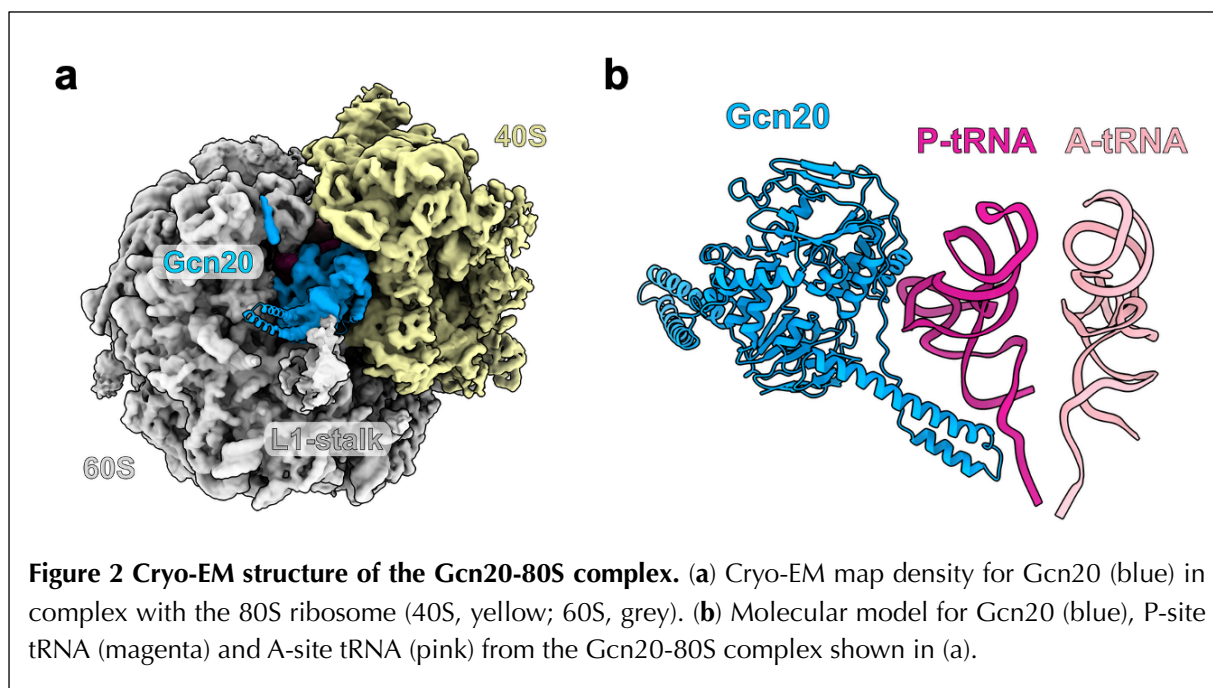
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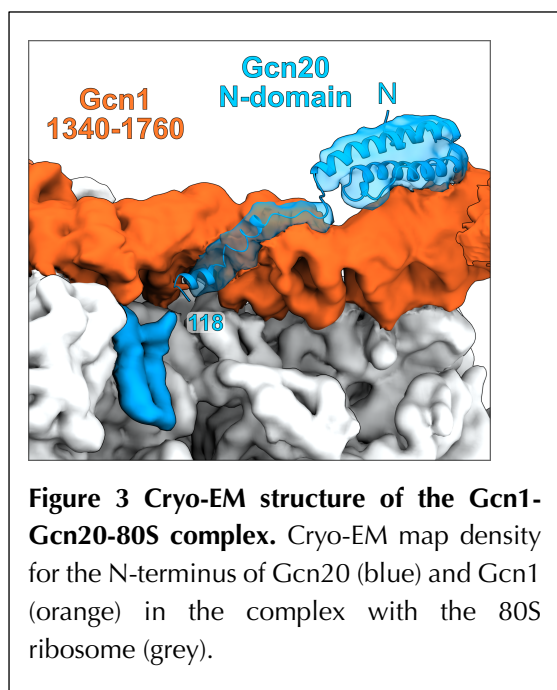
Joint Research Report (Annual/Project Completion)

Annual Report
Report
Two main objectives were achieved in (2025): 1. The first joint study from the Wilson and Inada groups was published in PNAS in 2025 (Paternoga et al Proc Natl Acad Sci U S A. 2025 Apr 15;122(15):e2415807122. doi: 10.1073/pnas.2415807122. Epub 2025 Apr 8. PMID: 40198700). Briefly, using ex vivo affinity purification approaches, the Wilson group could obtain a cryo-electron microscopy structure of a yeast Gcn2 dimer in complex with the ribosomal 60S subunit (Figure 1). We could show that the Gcn2 dimer is formed by dimerization of the histidine tRNA synthetase-like domains, which establish extensive interactions with the stalk-base and sarcin-ricin loop of the 60S subunit. The C-terminal domain of Gcn2 is also dimerized and occupies the A- and P-site tRNA binding sites at the peptidyl-transferase center of the 60S subunit. Complementary functional studies from the Inada group indicated that binding of Gcn2 to the 60S subunit does not require the coactivators Gcn1 or Gcn20, nor does it lead to phosphorylation of eIF2α. Instead, upon stress, we observe a shift of Gcn2 from the 60S subunit into the colliding ribosome fraction, suggesting that the Gcn2-60S complex represents an inactive stand-by state to enable a rapid redistribution to collided ribosomes, and thereby facilitating a quick and efficient response to stress. a Gcn2-60S CP L1 stalk L11 stalk 60S Subunit Gcn2 Dimer b Gcn2 1 137 276 539 585 1016 1058 1490 1533 1649 RWD ΨKinase Kinase (PK) His-RS CTD c CTD Dimer HisRS Dimer ΨKinase Domain PK Domain d CP L11 stalk CTD Dimer HisRS Dimer ΨKinase Domain PK Domain Figure 1 Cryo-EM structure of the Gcn2-60S complex. (a) Cryo-EM map density for Gcn2 (orange) in complex with the 60S subunit (grey). (b) Schematic representation of the domain structure of Gcn2, with RWD (grey), Ψ-kinase (green), PK (blue) domains, HisRS-like (HisRS, yellow) and C-terminal domain (CTD, pink). (c) cryo-EM map density for Gcn2 (transparent grey) with fitted domains, coloured as in (b). (d) Molecular model for Gcn2 (coloured by domain as in (b)) on the 60S subunit (grey). Landmarks L1 and L11 stalk and central protuberance (CP) as indicated.

2. Re-analysis of our original Gcn1-disome datasets that were derived from our first study (Pochpien et al Wilson (2021) Proc Natl Acad Sci U S A Apr 6;118(14):e2022756118. doi: 10.1073/pnas.2022756118.) led us to identification of the binding site of Gcn20 on the ribosome by the Wilson group. Specifically, we observe that the ABCF domain of Gcn20 binds within the E-site of the 80S ribosome (**Figure 2**).



Interestingly, the Wilson group could also identify an interaction between the N-terminal domain of Gcn20 and Gcn1 on a collided disome. In particular, we observe that Gcn20 contains a linker between the N-terminal domain and ABCF domains that makes extensive interaction with the 80S stalled ribosome within the collided disome (**Figure 3**).



These structural results were used to design a series of Gcn20 linker variants that were tested in collaboration with the Inada group to investigate the role of Gcn20 for Gcn1 interaction with 80S and disomes, as well as for Gcn2 activation (**Figure 4**).

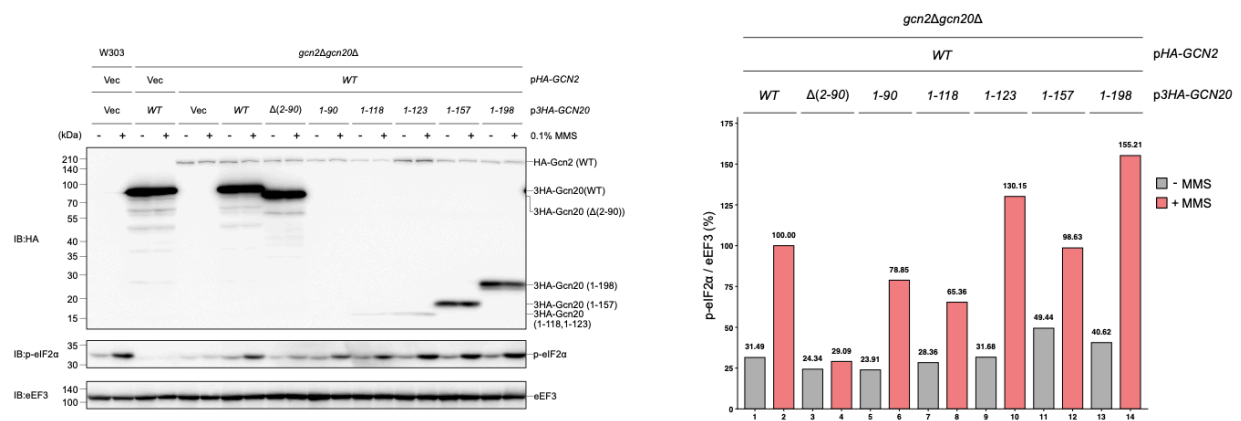


Figure 4 Biochemical characterization of N-terminal truncations of Gcn20 on eIF2α phosphorylation. Biochemical analysis of N-terminal truncations of Gcn20 on eIF2 phosphorylation (left) with quantitation (right). The data were produced by Taiga Okamoto from the Toshi Inada lab.