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研究課題名	Structures of the Gcn2 kinase on the ribosome	
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Joint Research Report (Annual/Project Completion)

Annual Report

Report

In the first year, we have determined the structure of Gcn2 in complex with the 60S subunit. Additionally, in collaboration with the group of Prof. Toshifumi Inada, we have been able to demonstrate interaction of Gcn2 with 60S subunit under non-stress conditions and movement of Gcn2 from 60S to disomes/polysomes in the presence of stress. Binding to the 60S subunit is not dependent on Gcn1, whereas binding to disomes/polysomes is Gcn1-dependent.
This study was recently published in PNAS (Proc Natl Acad Sci U S A. 2025 Apr 15;122(15):e2415807122. doi: 10.1073/pnas.2415807122. Epub 2025 Apr 8. PMID: 40198700)

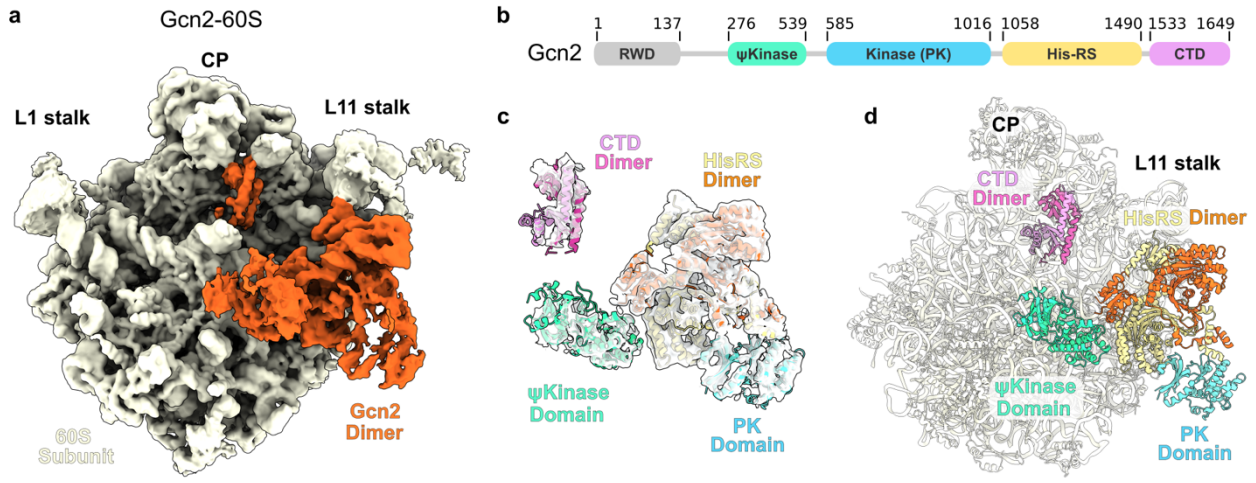

Figure 1 Cryo-EM structure of the Gcn2-60S complex. (**a**) Cryo-EM map density for Gcn2 (orange) in complex with the 60S subunit (grey). (**b**) Schematic representation of the domain structure of Gcn2, with RWD (grey), Ψ-kinase (green), PK (blue) domains, HisRS-like (HisRS, yellow) and C-terminal domain (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.

In summary, our study presents a structural and functional characterization of the interaction of Gcn2 with the 60S subunit. We observe that endogenous Gcn2 associates with the 60S subunit, consistent with many previous studies. However, unlike previous studies, we demonstrate that the interaction of Gcn2 with the 60S subunit occurs independently of the co-activator proteins Gcn1 or Gcn20. Moreover, we show that under stress conditions, such as MMS treatment, Gcn2 shifts from the 60S subunit to associate with disomes and polysomes, where collided ribosomes would be expected to be present. In the cryo-EM structure of the Gcn2-60S complex, we observe that the HisRS-like domain is a homodimer with an overall fold that is analogous to that of HisRS-like domain from yeast (Kluyveromyces marxianus) and human Gcn2 determined in the absence of the ribosome. The HisRS-like domain of Gcn2 has been reported to interact with tRNA, however, we did not observe density for tRNA bound to the HisRS-like domain of Gcn2 within the Gcn2-60S complex. This is not unexpected given that the complexes were not isolated under nutrient deprivation conditions that would lead to accumulation of deacylated tRNA. Nevertheless, we note that docking tRNA into the HisRS-like domain of Gcn2 bound to the 60S subunit based on its position in a HisRS-tRNA complex reveals that this placement would in principle be possible and not clash with the ribosome or any other domains of Gcn2. We observe density for one PK domain located near the N-terminus of the HisRS-like domain of one Gcn2 monomer, which forms interactions with the insertion subdomain of the HisRS domain of the same Gcn2 monomer. Unlike the HisRS-like and C-terminal domains, the PK domain is not dimerized in the Gcn2-60S complex. Although we observe additional density that may represent the second PK domain, the location

is distinct from the previously postulated inactive antiparallel or active parallel dimer conformations. Nevertheless, our biochemical results support an inactive state of the PK domain of Gcn2 within the Gcn2-60S complex, since we do not observe activation of eIF2α phosphorylation under conditions (absence of MMS) where the majority of Gcn2 is associated with the 60S subunit. Moreover, elevating the level of 60S subunits also did not lead to increased eIF2 phosphorylation or Gcn4 expression, suggesting that interaction of Gcn2 with 60S subunits does not lead to its activation. Curiously, we observed that mutations and deletions that were designed to reduce the interaction with the 60S subunit, also perturbed the association with collided disomes under stress (MMS) conditions. However, we note that the binding site of Gcn2 on the 60S subunit is incompatible with binding to a stalled 80S ribosome because of clashes between the ψPK, PK and HisRS-like domains of Gcn2 and the body of the 40S subunit. Similarly, although the CTD of Gcn2 would not clash with the 40S subunit, the binding position overlaps the binding site of peptidyl-tRNA at the P-site, which is observed to be present in previous structures of collided disomes. Thus, we favour a model where the conformation of Gcn2 on the 60S subunit represents an inactive stand-by state that becomes activated by stress due to the relocation of Gcn2 onto collided disomes, where a distinct ribosome binding mode is utilized.