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eIF5-mimic protein (5MP) is a translational regulatory protein that interacts with translation initiation factors and a competitive inhibitor of translation initiation. Expression of 5MP contributes to accurate translation initiation through preventing translation from non-AUG start codons. 5MP is known to be overproduced in certain types of cancer and promote the tumor growth. Moreover, preliminary studies indicate that 5MP is frequently overproduced in colon cancer and its expression correlates with the expression of the major oncogene, cMYC. How 5MP expression contributes to tumorigenesis is a mystery under intensive research.

We tested the model that 5MP's ability to suppress non-AUG translation contributes to carcinogenesis. For example, cMYC has two start codons, an authentic AUG start codon and an upstream in-frame CUG codon. The AUG-initiated isoform is oncogenic but the CUG-initiated form is not. 5MP expression promotes carcinogenesis by cMYC through repressing CUG-initiated isoform, while enhancing translation of the oncogenic AUG-initiated form.

We aimed to study the mechanism of 5MP repression of non-AUG translation. Human 5MP expression in yeast does not repress non-AUG translation, though it does so in human cells. We are finding that this is because human 5MP cannot bind yeast eIF3, a general translation initiation factor made up of 13 subunits in human and 6 subunits in yeast. By sucrose gradient fractionation technique, we observed an unusual co-sedimentation of human 5MP and yeast eIF5, which 5MP mimics, in the 48S ribosomal complex fraction. Because eIF5 antagonize accurate initiation, 5MP binding to the 48S complex would have otherwise removed eIF5 from the 48S complex by a competition mechanism. The co-sedimentation suggests that the inability of 5MP to bind yeast eIF3 prevents eIF5 from being evicted by 5MP. This in turn suggests that in human cells, the 5MP binding to eIF3, and subsequent eviction of eIF5 from the 48S complex makes initiation more accurate, hence suppressing non-AUG initiation.

The mechanical question was taken via yeast biochemical approach. The technique involves sucrose gradient velocity sedimentation, followed by western blotting of gradient fractions for proteins of interest. New plasmids were generated to express a 5MP model protein made from a part of eIF5, and see if this protein can outcompete eIF5 and make initiation accurate in yeast, in a manner dependent on the interaction with eIF3.