# Initiation of protein biosynthesis in Escherichia coli. Kinetics of 30 S initiation complex formation.

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The mechanism of 30S initiation complex formation and the effect of individual initiation factors and GTP on the process were investigated by stopped-flow kinetic measurements. The formation of the ternary complex was followed by an increase of both intensity and polarization of the fluorescence of a proflavine positioned in the anticodon loop of N-AcPhe-$tRNA^{Phe}$ from yeast (1,2).

In this way the process of initiation complex formation could be resolved into at least two partial reactions: a faster (relaxation time 0.1-1 s) apparent second-order reaction followed by a slower (relaxation time around 10 s) first-order reaction. The latter step is observed only in the presence of IF-2.

The effect of the initiation factors and GTP is to increase the velocity of ternary complex formation. The extent of this increase is $Mg^{++}$-dependent, being greatest at low concentrations of $Mg^{++}$ (400 fold at 7 mM $Mg^{++}$). The three factors and GTP seem to act synergistically.

The stopped-flow results complement and extend previous results obtained by the conventional filtration assay (3). A model describing the formation of the 30s initiation complex as a sequence of three consecutive reactions will be discussed.

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2) Robertson, J.M., and Wintermeyer, W. (1981) J. Mol. Biol. in the press.

3) Gualerzi, C., Risuleo, G., and Pon, C.L. (1977) Biochemistry 16, 1684-1689.