2.3 History and evolution of cell-free protein synthesizing systems

The very earliest studies of protein synthesis in cell-free systems included those of Siekevitz and Palade in the early 1950's. Palade's delightful account of how the foundations of cell biology were built is indispensable reading for anyone entering the field. The first of the modern cell-free protein synthesizing systems (circa 1970) evolved from the "readout" systems such as used by Redman et al. in the late 1960's. In these "zoo" systems purified large and native small ribosomal subunits (including bound initiation factors) were combined with "pH 5 enzymes" (aminoacyl tRNA synthetases and other factors) and energy generating systems, etc. WG (developed in the early 1970's) represented an advance in two ways over the zoo system. First, it was a single extract that contained all desired components, therefore much easier to prepare. It was also much more active in terms of both total synthesis, synthesis over background and rounds of translation per molecule of mRNA. Its disadvantage was that it gave a high proportion of "early quitters" and "false initiators" which appeared as a ladder of smaller bands down the gel. As a consequence of this it was a system good for only synthesizing relatively small proteins. Shortly thereafter the RRL system was popularized. The advantage of this system was that it was even more powerful than WG in terms of rounds of synthesis and in making full-length proteins. Two key obstacles were overcome in the early 70's that made it all the more attractive. The work of Hunt and others elucidated the hemin inhibited cascade of translational regulation which otherwise rapidly destroyed the activity of the lysates. Secondly, the introduction of micrococcal nuclease allowed endogenous mRNA (i.e. mainly globin mRNA) to be removed. Another problem with RRL remains to this day: the endogenous cold globin concentration is enormous (in the 100 mg/ml range). This limits the amount of total product you can analyze directly on a gel, makes sample preparation a headache (you need to use high concentrations of DTT to get complete reduction; TCA precipitation of undiluted lysate generates a precipitate with the key properties of cement), distorts both the gel and banding pattern, etc. An unanticipated advantage of RRL proved to be that protein translocation across the ER membrane proceeded with higher efficiency than in WG. In the meantime, the introduction of an organic solvent "flotation" step in the preparation of WG extract allowed the separation of "good" from "bad" embryos, presumably on the basis of their water content, that is those that were truly dormant embryos would float in a cyclohexane/carbon tetrachloride cocktail. These floated and dried embryos were found to make an extremely active protein synthesizing extract. Together with the newly introduced RNAse inhibitor from human placenta (RNAsin) this procedure placed the WG system on at least even footing with RRL for expression of high mw products with efficiency. Thus today, both systems have their place and value and their limitations. It is important to know these in order to make a rational choice of which system to use.

References:

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