Properties of ribosomes

0/ universal occurrence : amount correlated with protein synthesis.

1/ ~~Base~~ mainly RNA + protein ~ 60 : 40 in Coli
50 : 50 in higher organism.

2/ Contain most of the RNA of a cell.

3/ discrete sizes. Scheme for Coli depends upon Mg^{++} conc. ←?

$30S + 50S \rightleftharpoons 70S$ ~~$\rightleftharpoons$ 100S.~~

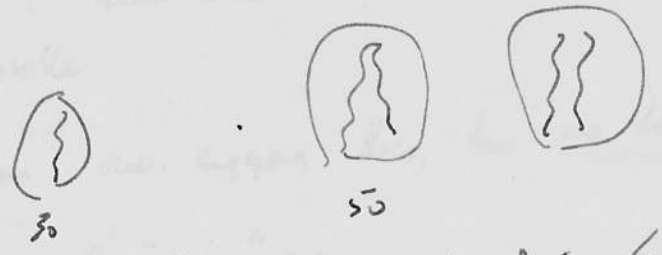
	30	50	70S	
10^6	1.0	1.8	3.1	?

$2\,70S \rightleftharpoons 100S.$

~~RNA~~

4/ size of RNA

~ 16S	$0.6 + 10^6$
~ 23S	$1.2 + 10^6$



composition of RNA (high G, med. A, C, low U.) | RNA shows some matching.
50i and 30i

5/ protein components. Same. 100 (histone)
comp. similar in all species.
subunits. eukaryotes (25,000)
liver, Ala + ...

6/ Shape of ribosome.

30S

Similar in other species ?

probably heavily hydrated.

Latent RNase on 30S particle (degrades to 2', 3')
cleaves to cyclic ~~3'~~ 2',3'
then to more slowly to 3' (some differ in rates for diff. bases)

~~amount~~ : ~~part~~ deals with ribosome breakdown ?

other latent enzymes ?

? are all ribosomes the same ? Theory of ribosome structure.

Nathans and Lipmann (PNAS) 1961 47 497

Amino acid transfer from aminoacyl-RNAs to protein on ribosomes of E. Coli

E. Coli used ~~loaded~~ aa-SRNA with Leu* to show transfer

to show requirement for a factor ribosomes must be "washed"
(spun 150,000 g for 3 hours)

One wash : shows some requirement

3 washes : show > abs. .... .

factor is non-dialysible : heat labile (1 min at 70°C)
rather unstable.

purified : some aa. enzymes lost, but no Leu activating activity.

appeared to be one factor for "all" a.a.

Since peak comes off DEAE-cellulose with same place
for transfer of Leu, Lys, Pro, Val and Tyr. (other claims for
not true by others)

Species specificity : previous work with rat liver factor had shown
no specificity { rabbit, pigeon, chicken or calf liver
or rabbit reticu. all could replace
rat supernatant.

but rat and E. Coli would not work (though E. Coli S-RNA used).
(Similar results by Rendi + Ochoa)

Conditions for transfer : Mg++ (.01 4M) optimal. GSH helps somewhat.
GTP needed . PEP + kinase needed.
inhibited by puromycin + chloramphenicol.
not reduced by cold Leu. (and true Leu* not incorp,
even if ATP included)

P.S. Leu that GTP split is ∝ aa incorp. two factors

~~recovered~~ recovered S-RNA appears uncharged

an enzymic deacylation reaction, needing ribosomes, ~~and~~ occurs in : with puromycin this is increased.
(also some loss by straight hydrolysis)

Occurrence of protein synthesis: how far is general scheme applicable?

nucleus: system similar to cytoplasm, except Na dependent
system to amino acid transport, and DNA (or other polyanion)
necessary for ATP supply.
believed to contain "ribosomes",
and activating enzymes and S-RNA (may be different)

cytoplasm: endoplasmic reticulum in some cells.
rarely in cells which secrete. (claim that ribosomes plus lipid do better).
"microsomes" use of deoxycholate to remove lipid, etc.
but ribosomes universal.
role of end. retic not clear, but suggests it
may help protein release. (Serum albumin in rat liver
can be released (after in vivo work
by DOC, or also ribosomes, from
microsomes, but not from ribosomes)
ie: Zeo

mitochondria:
do not take up aa* into "protein", but not into
cytochrome C or catalase. (RNase in same mitos.
needs energy supply)

mit. contain some 1% RNA.
if incorp done on intact mit. + then broken open,
most aa* counts in RNA-rich 100,000g sediment,
but no ribosomes seen so far.
acetone powder of mit. appears to contain aa. e.g. (PP^{+} exchange)
aa dep.

chloroplasts: some aa* incorp. RNA? prob. ribosomes??

General scheme. (having discussed props. of major components)

Act. enzyme.
S-RNA or T-RNA } ATP ~~+ GTP~~ (T-RNA acts catalytically)

~~GDP~~ GTP + transfer factor(s) + ATP generating system.

ribosome + messenger RNA — LATER.

release process? ATP dependent?

possible to get, in a cell-free system, synthesis (or finishing) of a well-defined protein.

remarks: dwell on specificity rather than on the biochemical steps.

"Special" Systems { early days.
rat liver. (serum albumin)
——→ (pea seedlings)

— E. coli.

— Reticulocytes Hb.