Control of ribosomal RNA synthesis in Escherichia coli

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CHAPTER VII

SUMMARY AND CONCLUSIONS

In this chapter we review our results and the most recent literature on the subject. Special attention is given to a comparison of in vitro and in vivo results on rRNA synthesis, for the ultimate goal of in vitro studies is to construct a rRNA synthesizing system containing all regulatory elements that are involved in vivo. As will be shown below, considerable progress has been made in that direction, although a number of questions remain to be answered.

The most obvious precondition for correct in vitro transcription is initiation and termination of the RNA chains at the right site. Studies of Pettijohn (1) and Jørgensen and Fiil (2) in different in vitro systems have shown that transcription occurs in the sequence 16S-23S which is the same as in vivo (3). Furthermore, the sedimentation constant of the rRNA made is approximately 30S (2) equal to that of the rRNA precursor in vivo (4). It can be concluded that rRNA transcription in vitro starts and ends at or near the in vivo sites. The final answer to this must come from sequence studies of the in vitro product and of the in vivo precursor.

One of the characteristic features of in vivo rRNA synthesis is the high initiation frequency of the RNA polymerase molecules on the rRNA cistrons which is 50-100 times that of the average mRNA cistron transcribed (5). As a result, up to 60% of all RNA made in fast growing E. coli cells is rRNA (see below) although less than 1% of the total DNA codes for rRNA. In purified systems only 2-10% rRNA synthesis is found (1, 6-9). The difference between the in vivo situation and the purified systems may be due to a low rRNA synthesis in vitro, for instance through loss of factors positively influencing rRNA synthesis on one hand or to an increased non-rRNA synthesis in vitro on the other.

We have studied this problem by using so-called nucleoids, the folded chromosomes, prepared by methods originally developed by Pettijohn et al. (10). These nucleoids were thought to be a more physiological template than DNA prepared by conventional procedures involving phenol extraction: the DNA in the nucleoids contains less breaks which may serve as artificial starting points for RNA polymerase (11) and has

a tertiary structure resembling the intracellular structure (12). The nucleoids could only be prepared in low concentrations which is an experimental disadvantage.

In Chapter IV (see also ref. 9) is shown that 57±4% of the RNA synthesized by RNA polymerase present in nucleoids from fast growing cells could be competed by rRNA from ribosomes. As expected, this is close to the in vivo value. Pato and Von Meyenburg (13) found that in fast growing cells 63% of all active RNA polymerase molecules is engaged in stable-RNA synthesis. This value was later corrected to 57% (see ref. 14). If we assume that 90% of the stable RNA is rRNA (14) than we arrive at 51% rRNA which is close to the value we found. It should be noted that about 20% of the in vivo rRNA precursor molecule is not conserved in vivo, indicating that in fast-growing cells about 60% of all active RNA polymerase molecules is engaged in rRNA precursor synthesis.

If RNA polymerase is added to the nucleoids it synthesizes rRNA at about the same rate as the endogenous enzyme but non-rRNA at a much higher rate resulting in a low percentage rRNA (3-5%) as demonstrated in Chapter IV. It is interesting that Giorno et al. (15) using nucleoids from slowly growing cells observed that the added enzyme synthesizes more rRNA than the endogenous one. Thus no special factor from fast-growing cells seems to be responsible for the high initiation rate on the rRNA cistrons.

In Chapter IV evidence has been presented that the initiation rate on the ribosomal cistrons is maximal in our systems; it is determined by the closely packed RNA polymerase molecules, i.e. the elongation rate. Since the elongation rate is only 10-20% of the in vivo value, the initiation rate of the RNA polymerase molecules on the rRNA cistrons in vitro is only 10-20% of the in vivo value in fast growing cells. We expect that an increased elongation rate in vitro will lead to an increased initiation frequency unless factors missing in purified systems are necessary for high rRNA initiation.

From our work with the nucleoids, we conclude that the percentage rRNA made in vitro is probably lower than the in vivo maximum for 2 reasons. Firstly, a decreased initiation rate on the rRNA cistrons due to a decreased elongation rate. Secondly, non-rRNA synthesis is much more abundant in vitro than in vivo under conditions of maximal growth.

Considerable evidence has accumulated that ppGpp is involved in the regulation of rRNA synthesis in vivo in E. coli and other procaryotes (16, see ref. 17). In earlier in vitro studies no specific inhibition of rRNA synthesis by ppGpp could be found (6,7,18). As pointed out in Chapter V this was probably partly due to the use of rather insensitive hybridization methods and partly to the use of unfavorable salt concentrations. With our highly sensitive hybridization system described in detail in Chapter III we could show that ppGpp specifically inhibits rRNA synthesis in purified in vitro systems containing DNA and RNA polymerase as the only macromolecular components (19,20, see also Chapters V and VI). This finding was later extended to crude systems (Chapter V). The most important findings concerning the specific inhibition of rRNA synthesis by ppGpp are:

(i) It is independent of the genome which the rRNA cistrons form part of. We found the effect with E. coli and $\lambda d_5 ilv$ DNA.
(ii) It was found with three different RNA polymerase preparations prepared in three different laboratories.
(iii) It is specific for ppGpp. Only pppGpp mimics the effect of ppGpp.
(iv) The K_i for the inhibition of rRNA synthesis by ppGpp is 0.15 mM.
(v) The inhibition is exerted at the level of initiation.

(i) and (ii) show that the ppGpp effect is not a special feature of a certain DNA and RNA polymerase preparation. Our finding in purified systems now has been confirmed by other investigators (2,21).

(iii) suggests that ppGpp itself and not a metabolite of ppGpp is the effector of rRNA synthesis in vivo, and shows that ppGpp does not act via substrate homology with GTP.

(iv) and (v) are in agreement with the findings on regulation of rRNA synthesis in vivo during amino acid starvation where it has been found that the apparent K_i for stable rRNA accumulation is 0.15 mM ppGpp (22) and that the inhibition is exerted at the level of rRNA chain initiation (23).

A comparison of in vitro and in vivo results strongly suggests that during amino acid starvation ppGpp alone is responsible for the sharp decrease in rRNA synthesis. It exerts its influence by inhibiting rRNA chain initiation by direct interaction with the RNA polymerase-ribosomal DNA system.

The regulation of rRNA synthesis during exponential growth and shifts in the growth rate is less well understood. Fiil et al. (22) have pointed out that the variation in ppGpp concentration as a function of exponential growth rate is much too small to suffice as the sole controlling element for the wide range in stable RNA accumulation observed. In amino acid starvation much larger variation in ppGpp concentration is correlated with equal changes in stable RNA accumulation. A similar conclusion was drawn from experiments performed during shifts in growth rate (24,25). Therefore, an additional control system for rRNA accumulation besides the control by ppGpp was proposed (22). Several possibilities exist for such an additional control such as: availability of RNA polymerase molecules (26), a positive effector of rRNA synthesis (22,24-26), rRNA breakdown (27), the phantom spot (28), or a factor influencing the interaction between ppGpp and RNA polymerase.

In vitro studies have only been concerned with positive effectors of rRNA synthesis. The existence of a positive control factor has been proposed by several investigators. Travers (7) suggested the elongation factor EF-Tu as a positive effector of rRNA synthesis. Unfortunately, attempts in several laboratories to reproduce his results have failed (2,6,20, see also Chapter V). In a recent study, Block (29) claims the existence of a factor in a crude system positively influencing rRNA synthesis. This factor however, has not been purified; his results may also be explained by changes in the RNA polymerase to DNA ratio in the reaction mixture. Thus the existence of a positive effector of rRNA synthesis is far from established. It is questionable whether such a factor is needed. Udvardy and co-workers (30,31) found that the interaction between RNA polymerase and the rRNA promoters is much stronger than the interaction between RNA polymerase and the average non-rRNA promoters.

The in vitro studies lead to the conclusion that two processes are part of the regulation of rRNA synthesis in vivo. These are the strong interaction between RNA polymerase and the rRNA promoters which allows a high initiation rate on the rRNA cistrons both in vivo and in vitro and ppGpp which decreases the affinity of RNA polymerase for these promoters.