PREFERENTIAL SYNTHESIS OF THE MESSENGER RNA OF THE HISTIDINE OPERON DURING HISTIDINE STARVATION

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Received November 18, 1968

The nine genes of the histidine operon of Salmonella typhimurium may be derepressed sequentially or simultaneously, depending upon the formylating capacity of the cell /Berberich et al., 1966, 1967/. Since the transcription product of the operon is a polycistronic messenger in both cases /Venetianer et al., 1968/ one must assume that during simultaneous derepression the whole operon is being transcribed before the translation of every cistron begins. This is in apparent contradiction with the hypothesis that translation and transcription are obligatorily coupled processes /Stent, 1964/. For this reason we wanted to investigate whether transcription of the histidine operon is possible in the absence of protein synthesis. Our experimental approach was to measure the biosynthesis of the histidine-messenger during amino acid starvation by means of hybridization to the DNA of the wild-type Salmonella typhimurium, and to the DNA of a deletion mutant in which more than two third of the histidine operon was missing.

Methods: Salmonella typhimurium LT-2 /wild-type/ and the histidine auxotrophs hisA30 /missense mutant/ and his152 /deletion in the histidine operon between genes D and F/ were provided by Dr. R.F. Goldberger. The tryptophan auxotroph try-4B was provided by the late Dr. M. Demerec. All the auxotrophs were derived from strain LT-2 and had a stringent control of RNA synthesis.

^{32}P labelled DNA was isolated from the LT-2 and the his152 strains by the method of Marmur /1961/. By mixing with unlabelled DNA the specific radioactivities were adjusted to such values which allowed the exact measurement of DNA without interfering with the tritium counting.

The auxotroph strains hisA30 or try-4B were grown in minimal salts-glucose medium /Vogel and Bonner, 1956/ containing limiting amounts of the required amino acid /2×10^{-5} M/. Under these conditions the bacteria grew exponentially until about 6×10^{8} cell/ml and then the growth abruptly ceased. Ten minutes later ^{3}H-uracil /usually 4 μC/ml 3.6 C/mM/ was added in some experiments together with 100 μg/ml chloramphenicol. After 15 minutes the pulse was terminated by chilling and centrifuging the cells and RNA was isolated by the phenol-SDS method.

Hybridization was carried out according to the method of Gillespie and Spiegelman /1965/. The RNA concentrations were not saturating /except in the case of ribosomal RNA/; the hybridization was nearly proportional with the input.

Radioactivity of the filters was determined in a Packard scintillation spectrometer. One channel registered the ^{32}P radioactivity without any contamination from ^{3}H, the second channel registered ^{3}H with 20 per cent efficiency and less than 1 per cent of the ^{32}P counts.

Results: Table I. summarizes the hybridization data. The amount of DNA on the filters was controlled by measuring the ^{32}P radioactivity. For the interpretation of the results it was essential to know that the two DNA-s were identical, except for the deletion in the histidine operon. This was established by hybridizing them with saturating amounts of labelled, purified 23s rRNA. The similar hybridization plateaus obtained in this experiment indicate that this is indeed the case, and the observed differences in the starvation experiments are meaningful. The results clearly show that if the histidine auxotroph hisA30 is starved for histidine, the hybridization of the labelled RNA to wild-type DNA is much higher than to the deletion mutant DNA. The difference is lower if chloramphenicol is present during the pulse /chloramphenicol increases the total incorporation into RNA more than tenfold/ but still considerable. On the other hand, no significant difference can be observed under conditions of normal exponential growth of the wild-type strain, or when the tryptophan-auxotroph try-4B is starving for tryptophan.

Compared to the elegant work of Imamoto et al. /1965/, with the tryptophan system, hybridization experiments with the histidine operon have a serious shortcoming. No specific transducing phage is available, and one has to work with the whole bacterial DNA. The length of the deletion in his152 is probably not more than 0.2 per cent of the genom, differences of this magnitude are well within the experimental error of the hybridization technique. Therefore it can be used only if the histidine-messenger is partially purified /Venetianer et al., 1968/ or if the relative amount of the labelled histidine-messenger is extremely high in the cell. Conversely one can

TABLE I.

SUMMARY OF THE HYBRIDIZATION DATA

RNA	DNA	RNA hybridized		Difference per cent
		dpm	per cent of input	
I/T-2, 23 s rRNA /100000 dpm/	20 µg I/T-2	666	0.67	-0.7
	" his152	671	0.67	
I/T-2, pulse-labelled during normal growth /167000 dpm/	50 µg I/T-2	10035	6.01	3.5
	" his152	9697	5.81	
hisA30, histidine-starved /40500 dpm/	5 µg I/T-2	491	1.21	103.0
	" his152	242	0.60	
hisA30, histidine-starved, +100 µg/ml chloramphenicol /171000 dpm/	15 µg I/T-2	1270	0.74	32.3
	" his152	960	0.56	
try-4B, tryptophan-starved /97000 dpm/	5 µg I/T-2	374	0.39	2.7
	" his152	364	0.38	

argue that when significant difference is observed in such a hybridization experiment, it indicates the presence of relatively large amounts of histidine-messenger. Thus we can conclude that under conditions of histidine starvation the relative amount of freshly synthetized histidine-messenger is extremely high /in the experiment shown it was more than half of the total hybridizable, labelled RNA. In some experiments this value was even higher/. It cannot be said whether histidine-messenger is synthetized in tryptophan-starved cells, but if so, its labelling is negligible compared to the other hybridizable RNA-s present.

Conclusions: The results reported here suggest that: 1/ Translation and transcription are not necessarily coupled. The synthesis of a specific messenger can be demonstrated in the absence of protein synthesis.

2/ Under conditions of histidine starvation an extremely large proportion of the synthetized RNA is the messenger of the histidine operon.

While this work was in progress, several papers have been published, showing that in E. coli the synthesis of the tryptophan-messenger occurs during tryptophan starvation /Edlin et al., 1968; Lavallé and de Hauwer, 1968/. Our results are in general agreement with theirs, except one important difference. According to our data the synthesis of the histidine-messenger during histidine-starvation is preferential over the other messengers. Therefore, we propose the hypothesis that the transcription of an operon responsible for the biosynthesis of an amino acid is specifically relieved from the general repression of RNA synthesis, imposed upon the cell by the lack of that particular amino acid. This would require more intricate regulation mechanisms than supposed earlier, but such mechanisms would be of obvious advantage to the economy of the bacterial cell.

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