The very similar kinetics of aminocacylation for these two tRNA molecules indicate that the modification of the side chain of ms\(^{20}\)A has a negligible effect on the recognition of E. coli tRNA\(^{20}\) by its homologous synthetase. This is consistent with the finding by Faulkner & Uziel (1971), who observed only a small loss of phenylalanine acceptance by I\(_2\)-treated E. coli tRNA\(^{20}\) in which ms\(^{20}\)A had been modified.

In view of the possible role of hypermodified nucleosides at position 37 in facilitating precise codon-anticodon base pairs (Nishimura, 1972), an investigation of the codon responses of this modified tRNA is planned.

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Early developmental changes in tRNA of Dictyostelium discoideum

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When vegetative amoebae of the slime mould Dictyostelium discoideum are starved they enter a series of developmental stages resulting in stalk cells and a fruiting body containing spores. Immediately on starvation (5 min after removal of nutrients), polyribosomes are degraded (Lodish et al., 1976). The mechanism that causes polyribosomal breakdown is a matter of speculation. During early and later development tRNA molecules are newly synthesized. At 2 min after deprivation of nutrients the ‘charging’ of tRNA\(^{20}\) is decreased by 70%, whereas several other tRNA molecules tested are ‘charged’ to the same extent as in vegetative cells. From this result we suggest that an ‘unloaded’ tRNA\(^{20}\) interferes with the elongation of peptide chains at polyribosomes and causes their breakdown. During preaggregation a tRNA\(^{20}\) isoacceptor accumulates (Dingermann et al., 1979). This tRNA contains the deaza-guanosine-derivative Q. The structure and modification of the tRNA\(^{20}\) isoacceptors of Dictyostelium discoideum is currently being investigated.

During vegetative growth 85% of the tRNA molecules that are present at polyribosomes comprise ribosylthymine at position 54, whereas tRNA molecules containing uridine at position 54 accumulate in the cytosol. During the early developmental preaggregation stage the amount of modified nucleosides in polyribosomal tRNA molecules is decreased. The polyribosomal tRNA molecules contain considerably lower amounts of 5-methyluridine, pseudouridine and 5-methylcytidine than polyribosomal tRNA molecules from vegetative cells. The developmental changes in tRNA modification and aminocacylation are assumed to be involved in the regulation of the synthesis of developmental proteins.


Restriction endonuclease EcoR1 binds non-specifically to deoxyribonucleic acid

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EcoR1 is a type-II restriction endonuclease (Boyer, 1971). These enzymes recognize and cleave specific DNA sequences. Despite the widespread use of these enzymes for genetic manipulation and DNA mapping, little is known about the mechanism of any of them, or how they achieve their specificity. EcoR1 recognizes and cleaves the DNA sequence

\[ 5'...G^AATTC...3' \]

at the point indicated (Hodgspeth et al., 1972). This activity occurs at around neutral pH and at salt concentrations above 500mM, but at lower salt concentrations and at pH values above 8, the enzyme exhibits EcoR1* activity when

\[ 5'...N^AATTN...3' \]

is cut (Polisky et al., 1975). Mg\(^{2+}\) is required for both activities. We have shown that EcoR1 not only binds to DNA containing EcoR1 or EcoR1* sites but will also bind to DNA containing neither site.

We have purified EcoR1 by modifying slightly the method of Green et al. (1978); this was followed by gel filtration through Sephadex G-100 (Greene et al., 1974). The enzyme has a specific activity of 88000 units/mg of protein; 1 unit being the amount of enzyme required to digest completely 0.5μg of plasmid-ColE1 DNA in 10μl of digestion buffer [100mM-Tris/HCl (pH 7.3)/50mM-NaCl/5mM-MgCl\(_2\)] at 37°C, in 15 min.

ColE1 is a circular plasmid (the preparation used contained a mixture of closed and open circles) with one EcoR1 site, and is therefore converted into a linear molecule by this enzyme. The linear form was separated from the open and closed circles by electrophoresis through a 0.8% agarose gel. The percentage digestion of the CoIE1 plasmid by EcoR1 was then determined by photographing the gel (stained with ethidium bromide) under u.v. light and then scanning the photographic negative at 550 nm in a Gifford spectrophotometer with gel-scanning attachment. The amount of DNA in each band on the gel was proportional to the intensity of its image on the photograph. This method was used to measure the initial rate of plasmid CoIE1 digestion by EcoR1 over a range of substrate concentrations. The data gave a \( K_m \) for plasmid CoIE1 of 3 nM. The turnover number at 37°C was 8 double-strand scissions/min per enzyme molecule.

We have investigated the effect of protein-modifying reagents on the activity of EcoR1. No loss in activity of the enzyme was observed after 1h with 12.5 mM-N-ethylmaleimide, or with 20mM-iodoacetate, or with 10mM-1-fluoro-2,4-dinitrobenzene.

1980
However, methyl acetimidate (a lysine-modifying reagent) was found to inactivate EcoR1. Incubation of the enzyme at 20°C with 33.33 mM methyl acetimidate resulted in complete loss of activity after 30 min whereas with 13.33 mM methyl acetimidate, only 60% of the initial activity was lost. We have found that DNA will protect EcoR1 from this specific inactivation. By measuring the protection as a function of DNA concentration, we have compared the binding of EcoR1 to DNA molecules containing EcoR1 and EcoR1* sites (SV40 DNA) with the binding to DNA containing neither poly(dA-dT). It was found that the binding to non-specific DNA is 10^4 times weaker than to EcoR1 or EcoR1* sites, and occurs both in the presence and in the absence of Mg^2+.

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**Properties of the deoxyribonucleic acid-binding site of *Escherichia coli* ribonucleic acid polymerase**

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RNA polymerase (EC 2.7.7.6) catalyses the transcription of RNA from DNA utilizing ribonucleoside triphosphates (Chamberlin, 1974). It has a mol. wt. of approx. 500 000, comprising five subunits in the stoichiometry αββ′σ (Burgess & Travers, 1971). Without the σ subunit, the core enzyme is still able to transcribe DNA, but lacks the specificity towards binding particular sequences that is observed with the holoenzyme (Chamberlin, 1974).

Because of their positive charge, lysine residues, of which there are approx. 205 in the core enzyme and 252 in the holoenzyme (Fujiki & Zurek, 1975), are likely to play an important part in the binding of DNA, and we have investigated their role in this function of core enzyme. This was performed by using the imido ester methyl acetimidate, which modifies protein molecules by covalently binding to the ε-amino group of lysine residues (Hunter & Ludwig, 1962). This particular reagent was chosen for a number of reasons: its specificity for lysine residues, its small size, the mildness of its reaction conditions, its retention of the positive charge after reaction with lysine, and the relative ease of its synthesis (Hunter & Ludwig, 1962; Bates et al., 1975). It is, however, unstable in aqueous solution, with a half life, at 25°C and pH 8.0, of 27 min (Hunter & Ludwig, 1962).

Preliminary experiments showed that the reagent inactivates core enzyme and renders it incapable of binding to DNA. However, it does not suffer any gross conformational change, as judged by its continued ability to bind rifampicin and to undergo limited proteolysis (P. A. Lowe, unpublished work).

Methyl acetimidate at 10 mM decreases the enzyme activity of core enzyme to between 5 and 10% of the control value within 90 min of treatment. If calf thymus DNA is included in the reaction mixture, some protection is observed, and, after the same time, 20–25% activity remains. Raising the salt concentration abolishes this protective effect of DNA. At higher reagent concentrations, even less activity remains, and the protective effect of DNA is diminished. Thus the lysine residues of the DNA-binding site are not the only ones essential for activity.

Because of aggregation of core enzyme at low ionic strength in the absence of DNA (Berg & Chamberlin, 1970; Smith et al., 1967; M. T. Record, personal communication), comparison between the number of lysine residues amidinated in the presence and absence of DNA was not meaningful. Instead, comparison was made between the number of amidinated lysine residues in the presence of DNA at high and low salt concentrations. After a correction had been made for a direct stimulatory effect of NaCl on methyl acetimidate, a difference of 15–20 lysine residues was obtained.

The concentration-dependence of the amount of amidination of core enzyme in the presence of DNA was investigated at high and low salt concentration. Kinetic analysis of these data suggested the existence of three classes of lysine residues. One class of 92 lysine residues could not be protected by binding to DNA. A second class of approx. 15 reactive lysine residues was also unprotected by DNA. A third class of approx. 13 lysine residues, protected by DNA, was found to be five to ten times more reactive than the unreactive lysine residues.

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