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Mutual Orientation of the Two L7/L12 Dimers on the 50S Ribosome of *Escherichia coli* As Measured by Energy Transfer between Covalently Bound Probes†

A. P. G. M. Thielen,* J. Antonie Maassen, Jan Kriek, and Wim Möller

ABSTRACT: The arrangement of the two L7/L12 dimers relative to each other on the *Escherichia coli* ribosome was studied by excitation energy transfer. We prepared four derivatives of these proteins with fluorescent probes bound at specific positions. *N*-[7-(Dimethylamino)-4-methylcoumarinyl]maleimide (DACM) was attached either to the N-terminal serine or to Lys-51 of L7/L12. Fluorescein, the energy-transfer acceptor, was introduced at the same positions. The modified proteins would still bind to the ribosome and remained functionally active in elongation factor dependent processes. Ribosomal particles were prepared with one dimer of DACM-labeled L7/L12 bound in the strong binding site [Zantema, A., Maassen, J. A., Kriek, J., & Möller, W. (1982) *Biochemistry 21*, 3077–3082]. Thereafter the second binding site was titrated with fluorescein-labeled L7/L12. From the resulting quenching of DACM fluorescence we calculated the distances between the probes in the four possible combinations. We find that at the N-termini the probes are 42 ± 5 Å apart and at the Lys-51 residues 66 ± 7 Å. The distance between the probes at the lysines-51 of the dimer in the strong binding site and those at the N-termini of the dimer in the weak binding site is 58 ± 6 Å. The other diagonal distance, i.e., between Lys-51 of L7/L12 in the weak site and the N-termini of L7/L12 in the strong site, is too long to be measured, that is, at least 60 Å. The problems arising from the elongated shape of an L7/L12 dimer and from the indirect coupling of two labels per dimer are discussed. Estimates are made of the distances between the two dimers at the different positions of the labels. It is concluded that the two L7/L12 dimers have separate binding sites on the ribosome, only one of which seems to be in the stalk of the 50S ribosomal subunit.

One intriguing aspect of ribosomes in general is that they contain a small acidic protein, which, opposed to most other components, occurs in multiple copies per ribosome (Matheson et al., 1980; Kurland, 1977). Their location on an easily recognized stalklike projection (Lake, 1976; Strycharz et al., 1978; Möller et al., 1983) and their interaction with soluble factors regulating the activity of the ribosomes (Möller, 1974) lend even more importance to this component. In *Escherichia coli* ribosomes it has been named L12 or, when the N-terminal serine is blocked by acetylation (Terhorst et al., 1972), L7. It is easily washed off from and rebound to the ribosome. In solution it forms elongated dimers (Möller et al., 1972; Wong & Paradies, 1974; Österberg et al., 1976). On the ribosome four copies (Subramanian, 1975), probably in the form of two dimers (Koteliansky et al., 1978; Zantema et al., 1982a), are present. For a recent review, see Liljas (1982).

The precise mechanism by which L7/L12 functions in protein synthesis is still unknown, and so is its exact location on the ribosome. For the study of topological aspects of large biological structures like ribosomes, the distance dependence of long-range nonradiative transfer of excitation energy between chromophores (Förster, 1967) provides a powerful tool (Schiller, 1975; Fairclough & Cantor, 1978; Epe et al., 1983). For such energy-transfer experiments an excellent donor–acceptor couple is formed by *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide (DACM)[1] and fluorescein. They show little overlap of their respective absorption and emission spectra and good overlap of donor (DACM) emission with acceptor absorbance (Zantema et al., 1982a,b).

In recent papers from our laboratory procedures are described to attach probes covalently to specific and well-defined sites on L7/L12; unlabeled L7/L12 could be removed by chromatographic methods (Zantema et al., 1982a; Maassen et al., 1983). Thus, we prepared the following 1:1 labeled proteins: DACM(1)L12 and Fluo(1)L12, L12 with either probe attached selectively to the N-terminus, and DACM-(51)L7 and Fluo(51)L7, in which the probes were attached with about 90% specificity to Lys-51 of L7.

The labeled proteins, like the unmodified ones, were still able to bind to L7/L12-depleted ribosomes and to restore their

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[1] Abbreviations: DACM, *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide; DACM(1)L12 and Fluo(1)L12, L12 with DACM and fluorescein N-terminally bound; DACM(51)L7 and Fluo-(51)L7, derivatives of L7 with the probes at Lys-51; EF-G, elongation factor G; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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activity. We prepared 50S ribosomes containing one dimer of DACM-labeled L7 or L12 and measured the quenching of DACM fluorescence induced by the addition of a second dimer labeled with fluorescein. From the results we estimated distances between the two L7/L12 dimers on the ribosome.

Experimental Procedures

Ribosomes were isolated from *E. coli* MRE600 according to Gesteland (1966) and separated into subunits by zonal centrifugation at low Mg^{2+} concentration (Möller et al., 1970). The 50S subunits lacking L7/L12, i.e., P_0 cores, were prepared by addition of cold ethanol up to 50% v/v to a 1 M ammonium chloride solution of 50S ribosomes at 0 °C (Hamel et al., 1972). Concentrations of ribosomes were determined from the absorbance at 260 nm; 1 A_{260} unit corresponds to 25 pmol of 70S, 39 pmol of 50S, and 69 pmol of 30S ribosomes.

Proteins L7 and L12 were isolated in 6 M urea (Möller et al., 1972) and labeled with DACM or fluorescein at either the N-terminus or Lys-51 as described by Zantema et al. (1982a) and Maassen et al. (1983). All preparations of proteins and ribosome were stored at −70 °C.

Protein concentrations were measured with insulin as a standard (Lowry et al., 1951); a molecular weight of 12 200 was used for L7 and L12 . After purification of fluorescein-labeled proteins by ion-exchange chromatography and by affinity chromatography of the SH-containing precursors of the DACM proteins, L7 and L12 bear one label per polypeptide (Zantema et al., 1982a; Maassen et al., 1983). This means that per dimer of L7 and L12 two fluorescein or two DACM groups are present. On the basis of the degree of labeling and the protein concentrations, we determined molar extinction coefficients of 85 000 at 495 nm for the fluorescein groups and 24 000 at 395 nm for DACM (cf. Yamamoto et al., 1977); these values are identical with the extinction coefficients of the free labels.

The dimer state of the modified proteins was verified by chromatography on a 144 × 0.9 cm column of Sephacryl S-200, which had been calibrated with unmodified (dimeric) L7 and with L7 monomerized by hydrogen peroxide treatment (Gudkov & Behlke, 1978). The flow rate was about 0.033 mL/min; the eluate was collected in fractions of 1 mL. In order to avoid possible interference of the monomeric material with the measurements of the activity of the reconstituted ribosomes and with the distance measurements, the L7 and L12 derivatives were routinely freed from monomers by the same gel filtration procedure.

EF-G was isolated according to Arai et al. (1972). Measurements of EF-G-dependent GTPase activity were performed as in Schrier et al. (1973). Whenever the term equivalents of L7/L12 is used, we refer to the number of L7 or L12 polypeptides. Hence, if 1 equiv of L7/L12 is added, on the average half a dimer of L7/L12 is present per ribosomal particle.

Repletion of P_0 cores was accomplished by incubation of the cores for 5 min at 37 °C or for 10 min at 25 °C with amounts of L7 or L12 as indicated in the figures. L7/L12–50S complexes were isolated by sedimentation (5 h at 55 000 rpm in a Beckman SW-60 rotor) through a 25% sucrose cushion. The buffer used in these and the energy-transfer experiments consisted of 20 mM Tris-HCl (pH 8.0), 10 mM magnesium acetate, 60 mM NH_4Cl, and 6 mM 2-mercaptoethanol. Absorbance spectra of the complexes were recorded on a Beckman Acta MVI spectrophotometer. After subtraction of the background due to the ribosomes, the concentration of labeled L7/L12 was determined from the absorption by the labels and the absorption coefficients given above.

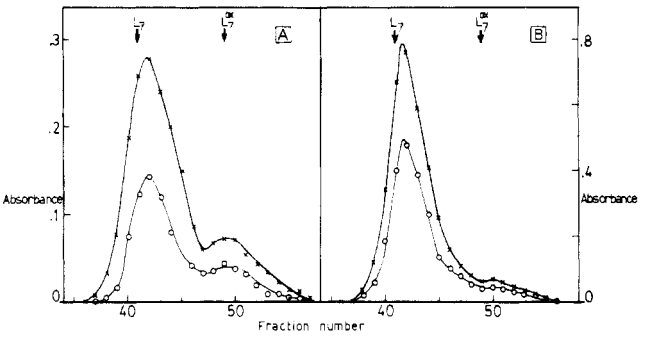

FIGURE 1: Gel filtration on Sephacryl S-200 of (A) 0.6 mg of DACM(1)L12 and (B) 1.5 mg of DACM(51)L7. Protein and DACM in the fractions were measured by their absorbance at 230 (×) and 395 nm (○), respectively. Dimeric L7 and monomeric, oxidized L7 eluted around the positions indicated by the arrows.

Fluorescence was measured at 22 °C in a Perkin-Elmer MPF-2A spectrofluorometer interfaced to an Apple IIE microcomputer. Two HN-38 polaroid filters obtained from Polarizers UK Ltd. were installed to avoid errors due to anistropy of emission (Badea & Brand, 1979). Absorbance at the wavelength of excitation was kept below 0.030. Bandwidths were set at 8 nm. The detector system of the apparatus was calibrated with the aid of a standard tungsten filament lamp, so that quantum-corrected emission spectra could be produced. Interfering emissions, e.g., scattering, were always corrected for by subtraction of the appropriate background signals. Quantum yields were determined relative to quinine sulfate in 0.1 N sulfuric acid, which has a quantum yield of 0.51 (Chen, 1973).

Anisotropy of emission was calculated from $(F_\parallel - F_\perp)/(F_\parallel + 2F_\perp)$; these broad-band emission signals were measured with the aid of the HN-38 polarizers mentioned above.

From the fluorescence data distances between specific points on the reconstituted 50S subunits were derived by using Förster's equation:

$$R = R_0(1/E - 1)^{1/6} \quad (1)$$

in which R is the distance between the labels, E is the fraction of excitation energy transferred, and R_0 is the distance at which 50% energy transfer occurs (Förster, 1967). In practice R_0 is determined from the spectroscopic properties of the donor–acceptor couple used [e.g., see Zantema et al. (1982b)]. In fact, R_0 also depends on the orientation of the transition moments of the probes with respect to each other, but this orientation factor (κ^2) is hard to measure directly. Therefore, it is usually assumed to be $^2/_3$. When the distances between the probes are being calculated, deviations from this situation with fully random orientation have to be taken into consideration. Limits for κ^2 and hence for R can be deduced from the emission anisotropies of the probes; to this end we used the results of Haas et al. (1978).

Results and Discussion

Properties of the Modified Proteins. By means of sedimentation equilibrium experiments unmodified L7/L12 and the fluorescein-labeled derivatives have been shown to form dimers in solution (Zantema et al., 1982a). From chromatography on Sephacryl S-200 we found that also the DACM-labeled L7 and L12 were mainly in the dimer form. Figure 1 shows that our preparations of DACM(1)L12 and DACM-(51)L7 contained about 20% and 10% monomeric material, respectively. Chromatography of Fluo(1)L12 and Fluo(51)L7 gave similar results (not shown). The higher amount of monomers present in the N-terminally labeled products may be

Table I: Spectroscopic Properties of the L7 and L12 Proteins Labeled with DACM and Fluorescein[a]

compound	absorption maximum (nm)	ϵ (mM^{-1} cm^{-1})	emission maximum (nm)	quantum yield	anisotropy
DACM(1)L12	395	24	478	0.16 (0.07)	0.13 (0.22)
DACM(51)L7	395	24	478	0.27	0.12 (0.17)
Fluo(1)L12	493	85	515	0.01	0.14 (0.17)
Fluo(51)L7	495	85	518	0.01	0.20 (0.24)

[a] All parameters are given for the free protein. Values which are different, when the proteins are bound to 50S ribosomes, are given in parentheses. Normally they refer to binding to the strong binding site; in the case of DACM(51)L7 and Fluo(51)L7 they appeared to be independent of whether the proteins were bound to the strong site or to the weak site.

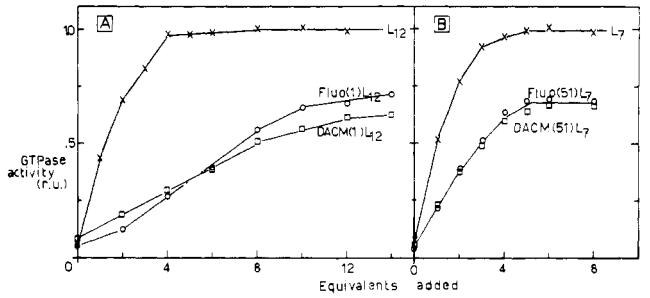

FIGURE 2: EF-G-dependent GTPase activity of P_0 cores in the presence of increasing amounts (A) of N-terminally labeled L12 and (B) of L7 labeled at position 51. The titration curves obtained with unlabeled L7 and L12 are shown for comparison. Amounts of protein added are given as equivalents of L7 or L12 per 50S core particle.

due to incomplete reversal by dithioerythritol of methionine oxidation by periodate. The periodate step is nececessary for the preparation of N-terminally labeled L12. Gudkov & Behlke (1978) have shown that oxidation of the methionine residues results in monomerizarion of L7/L12. In addition to the methionines, however, other elements most probably are involved in the process of dimerization. Although the presence of the probes in the N-terminal part of L12 may therefore affect the stability of the dimers, gel filtration shows that stable dimers of N-terminally labeled L12 are still formed. Before further use the monomers were removed from our preparations on Sephacryl S-200.

The EF-G-dependent GTPase activity of L7/L12-depleted ribosomes is very low. It can be restored by addition of L7/L12, a maximum being reached when about 4 equiv (i.e., two dimers) is added (Figure 2). The dependence of this GTPase activity on L7/L12 does not reveal any cooperativity in the binding and action of the two dimers. The shape of the curve obtained has been ascribed to a random occupation of two separate sites; the presence of only one dimer is sufficient for full activity of the ribosome (Möller et al., 1983).

In spite of the perturbation inevitably caused by the coupling of the probes to the protein backbone, the capacity to trigger EF-G-dependent reactions on the ribosome is to a large extent retained in the modified proteins, as can be seen in Figure 2. This result may be taken to indicate that in all our preparations the structure of L7/L12 is only little perturbed by the probes and the preparation procedures used. The maximal amount of restoration of GTPase activity appeared to be independent of the labeling site and the label used.

After modification of Lys-51, the maximal GTPase activity is still reached when about 4 equiv of the proteins is added (Figure 2B), suggesting that their binding properties are unaffected by the labels. In accordance with an earlier report (Zantema et al., 1982a), maximal restoration of activity by N-terminally labeled L12 (Figure 2A) required 10 equiv or more. This need for higher amounts indicates deteriorated binding of these proteins and illustrates once more that it is the N-terminal part of L7/L12 which is responsible for its binding to L10 and the 50S subunit (Koteliansky et al., 1978; Van Agthoven et al., 1975; Schop & Maassen, 1982).

A stable complex of P_0 cores and L7/L12 can be obtained by incubating the cores with excess L7/L12, followed by centrifugation through a sucrose cushion. When "undenatured" L7/L12 is used, four copies (two dimers) are bound to the ribosome. With L7 or L12 purified in 6 M urea the reconstituted complex normally does not contain more than 2 equiv (one dimer) bound. The plateau value of 2 equiv bound is reached when the cores are reconstituted with at least 4 equiv. These findings have been explained by the existence of two separate binding sites, a strong and a weak one for L7/L12 dimers on the 50S subunit (Zantema et al., 1982a,b). On addition of L7/L12 the two sites are occupied at random. If purified L7 or L12 is used, only the strong site remains filled in the absence of free ligand, e.g., on passage through a sucrose cushion.

More than 10 equiv of the N-terminally labeled L12 are required to saturate both binding sites (Figure 2). After isolation of the complex with the strong site occupied by one dimer, titration of the weak site was also less efficient with N-terminally labeled proteins compared with those labeled at Lys-51 [see below and Zantema et al. (1982b)].

The spectroscopic properties of the Fluorescein- and DACM-labeled products are given in Table I. The properties of the fluorescein-labeled proteins have been discussed before (Zantema et al., 1983a). The absorbance maxima of DACM(1)L12 and of DACM(51)L7 were at 395 nm. The maxima in the corrected emission spectra of both products were at 478 nm; no shift was observed upon binding of the DACM proteins to the core particles, which indicates polar environments for the probes on the ribosomes. When attached to Lys-51, DACM has a quantum yield of fluorescence of 0.27, both in solution and when bound to the strong site on 50S ribosomes. The emission anisotropy, in contrast, increased from 0.12 to 0.17 upon binding to the ribosome, indicating a decreased mobility of DACM at position-51 on the time scale of fluorescence. The mobility of the N-terminally bound DACM decreased even more upon binding; the anisotropy increased from 0.13 to 0.22. The quantum yield of DACM-(1)L12 dropped sharply from 0.16 in solution to 0.07 on the 50S ribosome. Both effects may indicate that the probe is rather strained by the environment in the L7/L12–L10 contact region of the large subunit where the N-terminus of L7/L12 is located. In this respect it is of interest that the fluorescence of N-terminally bound DACM on the ribosome is not quenched by fluorescein in solution or by fluorescein-labeled proteins that are not functionally bound to the ribosome (Figure 3). This behavior is indicative of a shielded enviroment, in which the probe is insensitive to the normally observed so-called aspecific quenching (Figure 4; Zantema et al., 1982b).

Determination of Distances between the L7/L12 Dimers on 50S Subunits. Complexes of 50S with a dimer of L7/L12 in the strong site were routinely prepared by incubation of P_0 cores with 8 equiv of the DACM-labeled proteins followed by

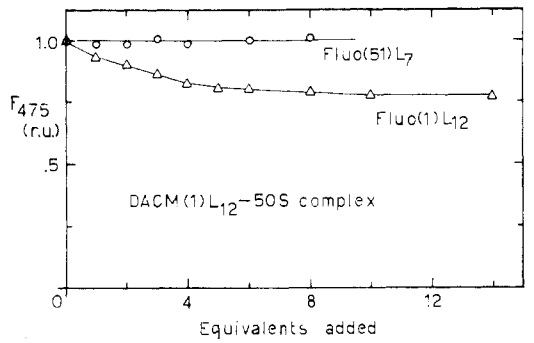

FIGURE 3: Effect of the addition of increasing amounts of Fluo(1)L12 and Fluo(51)L7 on the fluorescence of the DACM(1)L12–50S complex; average of three measurements; reproducibility within 4%. Although binding of Fluo(51)L7 must have occurred (Zantema et al., 1982b), no energy transfer is observed (upper curve). Filling of the weak site with Fluo(1)L12 required excess protein; finally 22% of the excitation energy is transferred from DACM to fluorescein in this case.

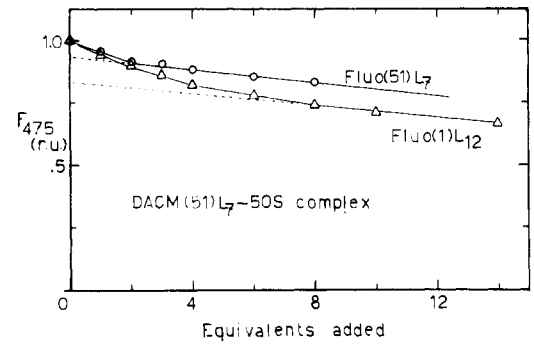

FIGURE 4: Titration of DACM fluorescence in the DACM(51)L7–50S complex; average of four measurements, reproducible within 4%. Upon addition of up to 2 equiv of Fluo(51)L7 a specific quenching is observed, indicating an energy transfer value of 0.09. Excess of this protein added quenches aspecifically (Zantema et al., 1982b). On titration with Fluo(1)L12 the results are somewhat more complicated. Since excess protein has to be added to completely fill the weak site, aspecific quenching competes with the specific quenching by the dimer bound to the weak site, throughout the titration. However, the aspecific quenching effects of Fluo(51)L7 and Fluo(1)L12 appear to be very similar, as can be seen from the slopes of the curves at the end of the titration. The two straight lines are shifted by 0.10 fluorescence unit. Thus, we find an energy transfer of 0.19 from DACM at position-51 to fluorescein at the N-terminus of the weak site dimer.

sedimentation through a sucrose cushion. Under these conditions occupation of the strong site by DACM(1)L12 was still incomplete (Table II, column 2). To reach similar DACM-(1)L12 to 50S ratios as with DACM(51)L7, twice as much DACM(1)L12 was required. Interpretation of energy transfer as measured by donor quenching, however, is not affected by the presence of ribosomes without donor-labeled L7/L12, simply because they are not seen by the fluorometer. Therefore, we did not saturate the complexes's strong sites with DACM(1)L12.

In Figures 3 and 4 the effect of titrating the weak site of the complexes with Fluo(1)L12 or Fluo(51)L7 is shown. With excitation at 395 nm and detection of emission at 475 nm mainly DACM fluorescence is monitored and the corrections for scattering and fluorescein emission are very small. In control experiments the fluorescence yield of DACM in the strong binding site appeared to be insensitive to the addition of unlabeled L7 or L12. When in a titration experiment the weak sites are all occupied by a fluorescein-labeled dimer, the fraction of donor molecules having acceptor molecules at their sides (Epe et al., 1983) equals 1. From the decrease of DACM fluorescence the degree of energy transfer between the labeled L7/L12 dimers on the ribosome was calculated as explained

Table II: Composition of DACM-Labeled L7/L12–50S Complexes.[a] Energy Transfer to Fluorescein-Labeled L7/L12 and Distances between the Labels

donor	DACM/ 50S[b]	acceptor	R_0 (Å)[c]	E[d]	R (Å)[e]
DACM(1)L12	1.1	Fluo(1)L12	34	0.22	42 ± 5
		Fluo(51)L7	34	<0.03	>60
DACM(51)L7	1.7	Fluo(1)L12	46	0.21	58 ± 6
		Fluo(51)L7	46	0.11	66 ± 7

[a] The complexes were prepared by reconstituting 50S core particles with 8 equiv of the DACM-labeled proteins and isolated by centrifugation through a sucrose cushion. [b] Determined from the 50S absorption at 260 nm and the DACM absorption at 395 nm. [c] Calculated assuming an orientation factor of $^2/_3$. [d] Energy-transfer values from Figures 3 and 4; if necessary corrected for the about 10% labeling of amino acids other than Lys-51. [e] The uncertainties imply experimental errors but mostly reflect the usual lack of knowledge of the actual value of the orientation factor. On the basis of the emission anisotopies of donor and acceptor, probability limits were estimated according to Haas et al. (1978).

in the figure legends. The results and the distances between the probes bound to the Lys-51 residues and the N-termini derived therefrom are listed in Table II. In the last column ranges for the probable distribution of the distances are indicated to account for the unknown value of the orientation factor κ^2; the limits were estimated from the emission anisotropies of the ribosome-bound labels (Table I) by the method of Haas et al. (1978).

The distances were calculated by a normal Förster treatment, i.e., for the situation in which the emission of all DACM molecules is equally quenched by one molecule of fluorescein. However, our system always contains two donor molecules and two acceptor molecules. General treatments of the case of multiple labeling have been given before (Gennis & Cantor, 1972; Epe et al., 1982). Since our system certainly does not represent a case of random labeling of a globular protein, it deserves special consideration.

The two Lys-51 residues as well as the N-termini are at least 30 Å apart in an L7/L12 dimer (A. P. G. M. Thielen, unpublished results). Consequently several possibilities for formation of donor–acceptor pairs must be taken into account. In Figure 5 a number of possible configurations are presented with their consequences for the calculation of distances from the energy-transfer results. The actual configuration is not known and may lie somewhere in between. In one extreme case (Figure 5B) both DACM molecules in the first dimer are equally quenched by the two fluorescein labels in the other dimer. The distance R then is about 12% longer (Gennis & Cantor, 1972) than that given in Table II. On the other hand, if only one of the two DACM moieties is sufficiently close to a fluorescein group (Figure 5C) to allow energy transfer to occur, the quenching observed is due to a double amount of energy transfer from only half of the DACM molecules. In this case recalculation of the shortest distance R between a donor–acceptor pair (Table II, column 5, and eq 1) yields values which are about 15% lower than the distances given in the last column of Table II.

The additional uncertainty of roughtly ±15%, resulting from the lack of information on the spatial orientation of the probes with respect to each other, has not been taken up in Table II, because we regard it as an interpretation uncertainty rather than as an experimental uncertainty. Besides, our aim was not to measure distances between chromophoric groups which are absent from a normal ribosome. Our objective was to study the arrangement of the two L7/L12 dimers on the ribosome with respect to each other and more specifically to investigate

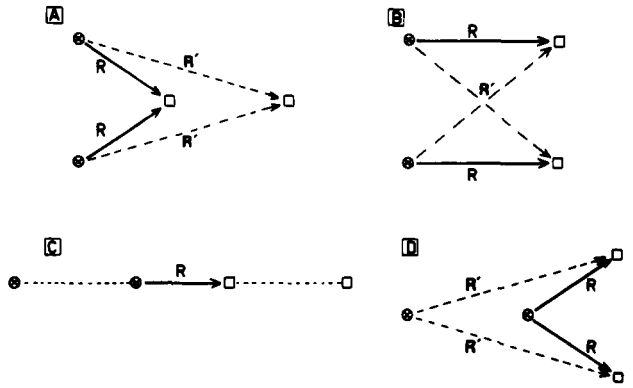

FIGURE 5: Schematic representation of possible orientations of the two DACM groups (⊗) and the two fluorescein groups (□) with respect to each other. For simplicity the 50S particle and the two L7/L12 dimers are not shown; the distance between the labels are representative for the situation on the reconstituted 50S ribosomes. R is the shortest distance between a donor and an acceptor; R' is a longer distance. (A) Both DACM molecules are quenched by one acceptor molecule; as to energy transfer this case is identical with the normal situation, i.e., with one donor and one acceptor. Hence, if R_F is the distance found from a normal Förster traeatment of the data, the ratio $R/R_F = 1$. (B) if R and R' are about equal, the acceptor absorbance at distance R is effectively doubled, and hence, $R/R_F = 2^{1/6} = 1.12$ (Gennis & Cantor, 1972). For $R' > R$ the ratio R/R_F lies between 1 and 1.12. (C) In this case the overall fluorescence yield measured is composed of unquenched emission from half of the DACM groups and quenched emission from the other half of the donors. So E, the actual energy transfer over the distance R, is twice the one measured. The ratio R/R_F then depends on E (cf. eq 1); for the distances we measured, $R/R_F \approx 0.85$. (D) This situation with half of the donor molecules feeling the presence of two acceptors contains features of both (B) and (C). As a result, R/R_F is about unity.

whether both dimers are close enough to each other to be fitted into the stalk of the 50S subunit.

The bifunctional reagent 4-(4-formylphenoxy)butyrimidate, used to attach fluorescein to Lys-51 (Zantema et al., 1982a), separates the probe by more than 5 Å from the protein backbone. In the other cases the probes are coupled directly (Zantema et al., 1982a) or cross-linked via a short propyl derivative (Maassen et al., 1983) to the protein. The relatively voluminous probes are unlikely to be buried inside the dimers. The probes bound to the lysines-51 most likely stick out on opposite sides of the L7/L12 dimer (A. P. G. M. Thielen, unpublished results). Taking into account in a coherent fashion the experimental uncertainties indicated in Table II, together with the possible positions of the probes relative to the L7/L12 dimers, and the various possible ways of donor–acceptor pair formation, we defined by trial and error the minimum distances between the two dimers which would be in accordance with the results of the measurements. In such a procedure the uncertainties in the mutual orientation of the probes and in the position of the probes with respect to the proteins do not simply add up. It appeared that on a reconstituted 50S ribosome the gap between the two L7/L12 dimers is at least 30 Å at the position of the N-termini and at least 45 Å at the Lys-51 residues. The shortest diagonal distance between the lysines-51 in the strong site and the N-termini in the weak site can be estimated to be at least 40 Å. Energy transfer along the other diagonal was not observed, which sets the lower limits of this diagonal distance between the dimers to about 50 Å. The maximum distances estimated in the same way were roughly 35% longer than those given in Table II.

Assuming a globular shape for L10 (M_r 18 000), the approximately 40 Å distance between the N-termini of the two dimers indicates that they bind on opposite sites of L10. From there on the elongated L7/L12 dimers apparently diverge, so

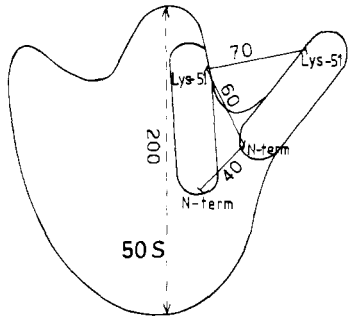

FIGURE 6: Hypothetical model of the location of the two L7/L12 dimers in the 50S ribosomal subunit. The distances measured in this paper were drawn on scale, assuming sizes for the 50S particle and an L7/L12 dimer of 200 and 100 Å, respectively. The position of the lysines-51 was chosen on the basis of the results of Maassen et al. (1984).

that at the position of lysines-51 they are 60–70 Å apart. It has been shown that the binding of only one of the dimers brings about the reappearance of stalks on the 50S core particles (Möller et al., 1983). The data presented here suggest that there can be no interaction between both dimers unless perhaps at the C-termini. Besides, if our reasoning is correct, our data virtually exclude the possibility that they are both located in the stalk, a morphological structure with a diameter of only about 40 Å (Lake, 1976).

In Figure 6 an attempt is made to fit the two L7/L12 dimers into a model of the 50S subunit by using the present and earlier data (Zantema et al., 1982b; Maasssen et al., 1984). Although the available cross-linking data (Traut et al., 1980) would give preference to a location of the second dimer in the central protuberance, as drawn here, we still lack definite information on its actual disposition on the ribosome, e.g., from immunoelectron microscopy [see Maassen et al. (1984) and references cited therein].

The distance measurements presented in this paper add detailed structural information on the L7/L12–L10 region of the 50S ribosome. Moreover, the experimental system provides tools for monitoring structural changes which may occur during protein chain elongation on the ribosome.

Registry No. GTPase, 9059-32-9.

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Probing the Conformation of 18S rRNA in Yeast 40S Ribosomal Subunits with Kethoxal†

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ABSTRACT: Yeast 40S ribosomal subunits have been reacted with kethoxal to probe the conformation of 18S rRNA. Over 130 oligonucleotides were isolated by diagonal electrophoresis and sequenced, allowing identification of 48 kethoxal-reactive sites in the 18S rRNA chain. These results generally support a secondary structure model for 18S rRNA derived from comparative sequence analysis. Significant reactivity at positions 1436 and 1439, in a region shown to be base paired by comparative analysis, lends support to the earlier suggestion [Chapman, N. M., & Noller, H. F. (1977) *J. Mol. Biol 109*, 131-149] that part of the 3'-major domain of 16S-like rRNAs may undergo a biologically significant conformational rearrangement. Modification of positions in 18S rRNA analogous to those previously found for *Escherichia coli* 16S rRNA argues for extensive structural homology between 30S and 40S ribosomal subunits, particularly in regions thought to be directly involved in translation.

Ribosomes from the cytoplasm of eucaryotes are distinguishable from those of procaryotic cells or organelles by their size, shape, and chemical composition (Wool, 1979). They are usually referred to as 80S ribosomes, dissociable into 40S and 60S subunits, in contrast with the 70S, 30S, and 50S subunits of procaryotes. They possess morphological characteristics that distinguish them from eubacterial or archaebacterial ribosomes (Lake et al., 1982) and have a higher content of protein relative to RNA (Wool, 1979). The large ribosomal RNAs of 80S ribosomes are also significantly larger than their eucaryotic counterparts; 18S rRNAs contain about 1800 nucleotides in contrast to about 1540 for procaryotes, while 26S or 28S rRNAs can have from 500 to over 1500 more nucleotides than the 2900 found in procaryotic 23S rRNAs (Noller, 1984). Thus, 70S and 80S ribosomes seem to represent two distinct classes.

Do the two classes represent fundamental differences in ribosome architecture, mechanism, and evolution, or are there underlying similarities between them? Sequence homology between yeast (Rubtsov et al., 1980), maize (Messing et al., 1984), *Xenopus* (Salim & Maden, 1981), *Dictyostelium*

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