Table 6 *Cis*-dominance of *uap*-100

Relevant genotype			Utilisation of (as nitrogen source) Hypoxanthine	Xanthine	Uric acid
Wild type			+	+	+
*are*A-102			+	−	−
uap-100	*are*A-102		+	+	+
uap-100 / +	*are*A-102		+	+	+
uap-100 / +	*uap*A-37 / +	*are*A-102 / *are*A-102	+	−	−
uap-100 / +	+ / *uap*A-24	*are*A-102 / *are*A-102	+	+	+

Two independent diploids were constructed for both *cis* and *trans* configuration dominance tests. Growth tests were carried out at 37 °C on solid minimal medium[26] with purines added at 100 μg ml^{-1}.

possibility. (2) *uap*-100 is a constitutive allele and *uap*A$^-$ mutations are noninducible alleles in a positive control gene. This is extremely unlikely as *uap*-100 and *uap*A$^-$ mutations do not affect the regulation of the enzymes induced together with the uric acid permease. Moreover, the strong 'up-promoter' effect would be unprecedented and difficult to accommodate to this model. (3) *uap*-100 is a mutation in a control region adjacent to a structural gene, *uap*A, coding for the uric acid–xanthine permease. This hypothesis is consistent with all the data and is definitely the most attractive.

Since the *uap*A gene is under the control of two positive regulatory genes, *ua*Y and *are*A, *uap*-100 can be described as an initiator constitutive mutation. Work is in progress to establish the nature of the interaction between *uap*-100 and the *ua*Y and *are*A products. Preliminary *in vivo* evidence indicates that *uap*-100 partially suppresses *ua*Y$^-$ mutations for permeation but does not alleviate the stringent requirement for the *are*A product. This latter finding is in essential agreement with the lack of effect of *uap*-100 on the ammonium-repressibility of the *uap*A permease. Therefore the suppression of *are*A-102 by *uap*-100 implies that *uap*-100, in contrast to its wild type allele, can accommodate to the modified *are*A product present in *are*A-102 strains. Probably the 'up-promoter' effect of *uap*-100 contributes to the magnitude of the suppression. 'Up-' and 'down-promoter' effects are a feature of many initiator constitutive mutations in the *ara* (ref. 21) and *mal* (ref. 22) systems of *E. coli*.

In addition to the *uap*A permease, *are*A-102 results in the loss of at least one other permease for uric acid and xanthine (H. N. Arst and C. Scazzocchio, unpublished). Although *are*A-102 leads to somewhat reduced levels of a number of enzymes and permeases[5, 17, 18, 23], these two (or more) permeases with overlapping specificities are the only activities which seem to be totally absent in *are*A-102 strains. This may be the first direct clue that similarity of binding sites for the *are*A product constitutes a physiologically significant form of control. The possibility that *are*A binding sites might exist in discrete classes related to each other by changes involving only a few bases is suggested by the observation (H. N. Arst and C. Scazzocchio, unpublished) that intracistronic reversions in an *are*A-102 strain yield new *are*A alleles with a phenotype mirroring that of *are*A-102. They result in recovery of uric acid and xanthine uptake activities but in loss of acetamidase.

Pairs of *are*A alleles with antisymmetrical properties and their respective initiator specific suppressors could well provide the means to identify and classify initiator sites in an eukaryote by purely genetical means.

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Determinant of cistron specificity in bacterial ribosomes

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The sequence of the 3′-terminus of 16S RNA from different bacteria has been determined. Complementarity relationships between this sequence and a purine-rich tract in the ribosome binding site of different bacterial mRNAs suggest that the 3′-end of 16S RNA determines the intrinsic capacity of ribosomes to translate a particular cistron.

INITIATION of protein synthesis in bacteria involves the specific binding of the small ribosome subunit to a region of the mRNA containing the initiation codon. As the initiating 30S subunit discriminates against the many internal AUG or GUG codons and selects only the AUG or GUG triplet at the beginning of a cistron, some feature of the mRNA other than the presence of AUG must be necessary for ribosome recogni-

Table 1 Determination of the 3′-sequence of *C. crescentus* 16S RNA

Number of stepwise degradations before labelling with ^{3}H-isoniazid	Radioactivity in nucleoside hydrazones released by pancreatic ribonuclease*				Sequence
	G-iNzd	U-iNzd	A-iNzd	C-iNzd	
0	3.5	83.8	8.1	4.6	PyU_{OH}
1	3.5	8.7	8.0	79.9	$PyCU_{OH}$
2	5.6	74.3	6.4	13.7	$PyUCU_{OH}$
3	8.3	70.4	9.9	11.3	$PyUUCU_{OH}$
4	6.0	71.8	5.1	17.1	$PyUUUCU_{OH}$
5	6.4	19.2	7.3	67.1	$PyCUUUCU_{OH}$
6	7.6	13.3	8.0	71.1	$PyCCUUUCU_{OH}$
7	8.6	62.9	11.4	17.1	$PyUCCUUUCU_{OH}$

C. crescentus (ATCC 15252) was grown and collected as described by Leffler and Szer[2]. RNA was extracted directly from the cell pellet with a mixture of phenol/cresol-aminosalicylate[35]. DNA, low molecular weight RNA and polysaccharide were removed by washing with 3 M sodium acetate[35]. 16S RNA was isolated on 5–20% (w/v) sucrose gradients. After 3′-terminal labelling, 16S RNA was digested with pancreatic ribonuclease (100 μg per mg RNA) at 20° C for 4 h in 0.01 M Na/K phosphate buffer (*p*H 7.4). A sample of the digest (10 or 20 μl) was mixed with 20 μl of unlabelled mononucleoside hydrazones[39] and electrophoresed on Whatman 3MM paper at 40 V cm^{-1} for 2 h in 0.1 M sodium formate (*p*H 3).

*Expressed as a percentage of total radioactivity in nucleoside hydrazones separated by paper electrophoresis.

tion. Such recognition probably involves some as yet undefined component or feature of the untranslated sequence on the 5′-side of the initiator codon. After the specific interaction of mRNA with the 30S subunit, stabilisation of the initiation complex is dependent on initiation factors[1-3], and possibly on the 'fractional' ribosomal protein S_1 (ref. 4).

Cistron specificity

A number of reports demonstrate that ribosomes from different bacterial species show cistron specificity in the translation of natural mRNAs *in vitro*, irrespective of the source of the initiation factors used[2,5-11]. Thus, ribosomes from *Micrococcus cryophilus* or *Pseudomonas* translate protein from all three cistrons of MS2 RNA in the same relative amounts as those from *Escherichia coli*[8]. Under comparable conditions *Bacillus stearothermophilus* ribosomes translate only the A-protein cistron of R17 or f2 RNA (refs 5–7). *Caulobacter crescentus* ribosomes are unable to translate any of the MS2 RNA cistrons[2]; correspondingly, *E. coli* ribosomes do not translate RNA from the *C. crescentus* phage Cb5 (ref. 2). A similar cistron selectivity is also demonstrated, in the absence of initiation factors, in a weak, but species-specific interaction of ribosomes with certain phage RNAs (ref. 4).

Studies with hybrid ribosomes show that the specificity of ribosome binding and translation of natural mRNAs is primarily a function of the 30S subunit[2,7]. Two recent reports aimed at determining the component of the 30S subunit responsible, argue that both a ribosomal protein (particularly protein S12) and the 16S RNA determine the specificity of initiation[12,13]. The type of recognition process involved, however, is unknown.

Base sequence or secondary structure?

The recognition of mRNA initiation signals by 30S subunits could conceivably be determined by a specific base sequence on the 5′-side of the initiation triplet, by the degree or type of secondary structure in this region of the mRNA, or by both factors. Ribonuclease digestion of initiation complexes formed on specific mRNAs has made it possible to determine the base sequence of ribosome-binding sites for several mRNA cistrons[6,14-23]. Although some of these ribosome-protected sequences probably have a fairly high degree of secondary structure[6,14,19,24], no such secondary structure seems possible for other initiation sites[23,25,26]. It therefore seems unlikely that the 30S subunit specifically recognises some feature of the secondary structure of the ribosome-binding site sequence, particularly since a reduction in structure on partial denaturation of intact mRNAs generally increases, rather than decreases, the number of available initiating sites[27,28]. This observation therefore suggests that the specific binding of ribosomes to mRNA may depend on the ribosome-binding site being in an open, single-stranded form, rather than in a structured conformation.

It is significant therefore that all coliphage RNA ribosome-binding sites examined to date contain all or part of the purine-rich sequence 5′-AGGAGGU-3′ in a similar relative position on the 5′-side of the initiator triplet AUG. The ribosome binding site of an endogenous *E. coli* mRNA also contains part of this sequence, AGGA (ref. 23). We have previously shown that the 3′-terminus of *E. coli* 16S RNA contains the sequence 5′-ACCUCCU-3′ which is the complement of AGGAGGU (ref. 29). This, together with evidence that an intact 3′-terminus of 16S RNA is necessary for protein synthesis in bacteria[30-32] and more recent data suggesting a specific role for the 3′-terminus in initiation[33], have prompted us to suggest that the 3′-terminal sequence of 16S RNA may have a direct base-pairing role in the initiation of protein synthesis on natural mRNAs (ref. 29).

This hypothesis predicts that there will be a positive correlation between the translation of a particular bacterial mRNA cistron by bacterial ribosomes, and the degree of complementarity which exists between the ribosome binding site sequence of that cistron and the 3′-terminal sequence of the 16S ribosomal RNA. We have therefore determined the 3′-terminal sequences of 16S RNA from *P. aeruginosa*, *B. stearothermophilus* and *C. crescentus* since information exists on both the capacity of ribosomes from these bacteria to translate various bacterial mRNA cistrons, and on the ribosome binding site sequences of the cistrons concerned.

3′-terminal sequences

A procedure for the stepwise removal of seven to eight nucleotides from the 3′-terminus was applied to 16S RNA isolated from bacteria (see Tables 1 and 2). This is a cyclic process and involved removal of the periodate-oxidised 3′-nucleoside by incubation in 0.33 M aniline, digestion with alkaline phosphatase to release the 3′-phosphate and periodate-oxidation of the resultant 2′,3′-hydroxyl groups[29,34]. After removal of successive 3′-nucleosides, the RNA was labelled by condensation with ^{3}H-isoniazid[35] and digested with specific nucleases. The labelled 3′-terminus was characterised by chromatography of the ribonuclease digest on DEAE-Sephadex and identification of any 3′-nucleoside hydrazones by paper electrophoresis[36,37].

An outline of the sequence determination for *C. crescentus* 16S RNA is shown in Table 1. The 3′-sequences of 16S RNA from the other bacteria, determined by the same method, are listed in Table 2. A general feature of all the 3′-termini is the high proportion of pyrimidine residues. Apart from the terminal adenosine, the seven terminal nucleotides at least, are invariably uridylic acid or cytidylic acid.

Complementarity to coliphage ribosome-binding site sequences

We have previously shown that the 3′-terminal nucleotides of *E. coli* 16S RNA could form seven, four and three base pairs with the appropriate region of the R17 A-protein, replicase and coat-protein ribosome-binding sites, respectively (see Table 3 and ref. 29). The extent of this pairing relates closely to the ribosome-binding capacities of the three isolated initiator regions of R17 RNA since *E. coli* ribosomes discriminate in favour of the A-protein initiator fragment some fortyfold and elevenfold over the coat and replicase sites, respectively[38]. This is in contrast to initiation of the three cistrons on intact R17 RNA where some 20 moles of coat protein and 5 moles of replicase are synthesised for each mole of A-protein[44]. Under these conditions the secondary structure of the intact phage RNA presumably impedes binding of the 30S subunit to the A-protein and replicase initiator regions[38].

The 3′-end of *Ps. aeruginosa* 16S RNA is identical to that of *E. coli* for seven nucleotides; it can be aligned such that five, four and three to four base pairs are possible with the ribosome-binding site sequences of the A-protein, replicase and coat-protein cistrons of R17 RNA, respectively (Table 3). It has been previously shown[8] that *Pseudomonas* ribosomes translate all three cistrons of MS2 RNA in a manner analogous to that of *E. coli* ribosomes.

B. stearothermophilus (and *B. subtilis*) 16S RNA has a 3′-sequence significantly different to that of *E. coli* and *Ps. aeruginosa* 16S RNA (Table 2). Some complementarity ($\geqslant$ four base pairs) exists, however, between this sequence and the ribosome-binding sites of both the A-protein and replicase cistrons of R17 RNA (Table 3). The degree of complementarity possible with the coat-protein binding site is considerably less (one to two base pairs). *B. stearothermophilus* ribosomes bind significantly only to the A-protein site on native f2 or R17 RNA (refs 6 and 7). Appreciable translation by *B. stearothermophilus* ribosomes of both the replicase and A-protein cistrons does occur, however, after unfolding of the RNA with formaldehyde[28]; under these conditions no coat protein is synthesised. *B. stearothermophilus* ribosomes also exhibit a relatively high level of recognition of the R17 replicase initiation site when assayed at 49 °C (ref. 13); the T_m of the short helix containing the R17 replicase initiator region is 48 °C in 0.05 M Na^+ (ref. 24). *B. stearothermophilus* ribosomes are therefore intrinsically able to recognise the A-protein and replicase sites but not the coat-protein site, as would be predicted if a minimum of three to four base pairs were necessary to permit stable interaction. Presumably the conformation of the native, intact phage RNA usually renders the replicase site inaccessible to *B. stearothermophilus* ribosomes.

B. stearothermophilus ribosomes bind to only a single site on Qβ RNA at 65 °C, which does not correspond to any of the three normal initiator regions[26]. The ribonuclease-protected fragment is similar in size to that bound by ribosomes during productive initiation on the A-protein cistron of R17 RNA. It contains no initiation triplet, showing that mRNA can be specifically recognised and bound by ribosomes independently of polypeptide chain initiation[26]. The fragment, however, contains a polypurine tract of composition G(AAAG,AG,G,G,G)A in a similar relative position to that of AGGAGGU in the R17 A-protein site[26]. It is significant therefore that the complement of the 3′-terminus of *B. stearothermophilus* 16S RNA is AGAAAGGA.

As the 3′-sequence of *C. crescentus* 16S RNA is similar to that of *B. stearothermophilus* 16S RNA (Table 2), one could predict a similar recognition by *C. crescentus* ribosomes of both the A-protein and replicase cistrons of R17 RNA (Table 3). This prediction is not inconsistent with the observed failure of *Caulobacter* ribosomes to translate MS2 RNA at 37 °C, as under these conditions the initiation sites for the A-protein and replicase cistrons are presumably largely masked by the secondary structure of the phage RNA (refs 24 and 38). This is analogous to the very low level of translation of f2 RNA by *B. stearothermophilus* ribosomes at 37 °C compared to that found at 65 °C or after mild denaturation of the RNA with formaldehyde[28]. It is significant that considerable translation of formaldehyde-treated MS2 RNA occurs with *Caulobacter* ribosomes[2], although the identity of the translated cistrons is not known.

The degree of possible interaction between the 3′-terminus of 16S RNA from several different bacteria and the ribosome-binding sites of coliphage RNA is therefore consistent with the available data on translational specificity in these bacteria. Confirmation of the hypothesis that the 3′-end of 16S RNA is directly involved in the selection of initiation regions on mRNA must await the sequencing of ribosome-binding sites from other bacterial messengers and direct demonstration of such an interaction. For instance, we would predict that the sequences of the ribosome-binding sites from RNA of the *C. crescentus* phage Cb5 would contain some part of the sequence complementary to the 3′-end of *C. crescentus* 16S RNA, that is, some part of AGAAAGGA.

Table 2 3′-terminal sequences of 16S rRNA

E. coli B	$GAUCACCUCCUUA_{OH}$ (ref. 29)
P. aeruginosa	$G(X)_2PyCUCUCCUU(A)_{OH}$*
B. stearothermophilus	$G(X)\sim_5PyUCCUUUCU(A)_{OH}$*
B. subtilis	$G(X)\sim_7PyCUUUCU_{OH}$
C. crescentus (ATCC 15252)	$G(X)_3PyUCCUUUCU_{OH}$

Bacteria in mid-log phase were converted to protoplasts and extracted with a solution of phenol/cresol-aminosalicylate (see ref. 29 and legend to Table 1). For all bacterial species examined, chromatography on DEAE-Sephadex of T_1-ribonuclease digests of terminally-labelled 16S RNA demonstrated the presence of a large ($\geqslant$12 nucleoside residues) 3′-terminal T_1-oligonucleotide. X represents any nucleoside other than guanosine. A more detailed account of the sequences and their determination is in preparation.

*The variable presence of the 3′-terminal adenosine in 16S RNA is found in a variety of bacteria and depends on the culture conditions. A more extensive examination of this phenomenon will be the subject of a separate report.

Initiation factors

The role of initiation factors (for example, IF-3) in determining the efficiency of initiation on certain cistrons or mRNAs seems to be related to their capacity to stabilise the mRNA-30S subunit complex[1-3]. 30S subunits, possibly through the 3′-end of 16S RNA and protein S12 (ref. 12), have an inherent capacity to bind weakly but specifically to mRNA (ref. 4) and are stabilised in this interaction by various initiation factors, particularly IF-3 (refs 1–3) and the ribosomal protein S_1 (ref. 4).

The role of IF-3, which interacts with both 30S subunits and with mRNA (refs 1–3, 45), could be to potentiate or stabilise base pairing between the 3′-terminus of 16S RNA and the complementary region of the ribosome-binding site. Purified IF-3 species promote differential translation of coliphage RNA cistrons[46-48]; this may reflect a greater effect of IF-3 in the stabilisation of the mRNA–30S subunit complex at particular initiation sites. Thus initiation of the coat-protein cistron of R17 RNA, where only three to four base pairs can be formed between mRNA and the 3′-end of *E. coli* 16S RNA, may be highly dependent on the stabilising presence of IF-3 compared to initiation at the A-protein cistron, where seven such base pairs are possible. Such a view is consistent with the effect of IF-3 on ribosome binding to unfolded MS2 RNA, where addition of IF-3 stimulates ribosome binding to the coat-protein site but not to the other sites[48]. Similarly, crude initiation factors produce a significant stimulation in the binding of high-salt-washed ribosomes to the R17 initiator regions. This effect is about elevenfold, eightfold and fourfold for the coat-protein, replicase and A-protein sites, respectively[26].

Table 3 R17 ribosome-binding site sequences[6] and proposed pairing with the 3′-terminus of 16S RNA

Bacteria	R17 cistron	Possible pairing of 3′-terminus with appropriate region of ribosome-binding site sequence*		Number of base-pairs possible	Ribosome binding to unfolded R17, MS2 or f2 RNA (refs 2, 6, 8, 27, 28, 38)
E. coli B		$_{OH}$AUUCCUCCACPy	16S RNA (ref. 29)		
	A-protein	(5′) CU[AGGAGGU]UU(3′)		7	+
	Replicase	ACAU[GAGG]AUU		4	+
	Coat protein	ACC[GGG][G]UUUG		3(4)††	+
		↓ [A]†			
Ps. aeruginosa		$_{OH}$AUUCCUCUCPy	16S RNA		
	A-protein	(5′) CU[AGGAG]GUU(3′)		5	+
	Replicase	UG[AGGA]UUAC		4	+
	Coat protein	ACC[GGG][G]UUU		3(4)	+
		↓ [A]†			
B. stearothermophilus		$_{OH}$AUCUUUCCUPy	16S RNA		
	A-protein	(5′) AUCCU[AGGA]$\bar{G}$(3′)		⩾4	+
	Replicase	ACAUG[AGGA]U		4	+
	Coat protein	AACCGG[GG]UU		2(1)	−
		↓ A†			
C. crescentus		$_{OH}$UCUUUCCUPy	16S RNA		
	A-protein	(5′) UCCU[AGGA]$\bar{G}$(3′)		⩾4	See text
	Replicase	CAUG[AGGA]U		4	and ref. 2
	Coat protein	ACCGG[GG]UU		2(1)	
		↓ A†			

*The sequence given represents that containing all or part of the conserved sequence 5′-AGGAGGU-3′ from the A-protein, replicase and coat-protein initiator regions of R17 RNA. The initiator AUG is located eight to nine bases to the 3′-side of this sequence. Apart from the G→A transition (see below), these sequences are identical to those available for the corresponding regions of f2 (ref. 15) and MS2 RNA (refs 18, 21, 22 and 40).

†Two sequences have been reported for this region of the coat-protein binding site in the two different R17 stocks used[6,41]; the G→A transition probably represents a spontaneous mutation which occurred after the two stocks were separated[41]. Such a transition may have some selective advantage since it would increase the stability of interaction between the coat protein initiation site and the 3′-end of 16S RNA. The corresponding sequence from MS2 (refs 22 and 40) and f2 RNA (ref. 15) contains the A substitution. The *in vitro* ribosome-binding data were obtained with R17 RNA containing the GGGG sequence[6,38] and with f2 or MS2 RNA presumably containing the GGAG sequence[2,8,27,28].

‡The apposition of a G and a U residue in the proposed helical region formed between the coat-protein binding site and 16S RNA would cause little, if any, destabilisation of the base-paired structure[42,43].

The bacterial protein, factor *i* (interference factor), also modifies cistron selection in the translation of coliphage messengers by bacterial ribosomes. Significantly, it seems to be identical to the fractional 30S ribosomal protein S_1 (ref. 49), which can be linked chemically to the 3′-terminus of 16S RNA *in situ*[50] and which is necessary for the specific, initiation factor-independent binding of 30S subunits to mRNA (ref. 4).

Factor *i* is also identical to the α-subunit of the Qβ replicase complex[51]; the presence of the α subunit is essential for the binding of the replicase to the 3′-terminus of the Qβ+strand[52]. The 3′-sequence of this RNA is UCUCCUCCCA$_{OH}$ (ref. 53), and the 3′-sequence of 16S RNA is UCACCUCCUUA$_{OH}$, that is, they both share the sequence CCUCC which we propose to be involved in interaction with ribosome-binding sites. As factor *i* has a specific affinity for certain polypyrimidine tracts in RNA (ref. 54), its complex regulatory function may relate to a capacity for recognising such nucleotide sequences in mRNA and at the 3′-terminus of 16S RNA.

The inhibitory activity of factor *i* on the translation of coliphage RNA is restricted to initiation at the coat protein cistron[55] and is a function of mRNA concentration[54-,56]. This effect may be the result of both a direct binding of factor *i* to a region of the mRNA close to the coat protein initiation site[56], particularly at high concentrations of factor *i*, or to a competition between the factor and mRNA for a ribosomal site[54], possibly the pyrimidine-rich 3′-end of 16S RNA. It has recently been proposed that factor *i* bound to the ribosome may be directly displaced by added mRNA (ref. 54), the efficiency of which may depend on the sequence of nucleotides on the mRNA to which the ribosomes bind. Thus initiation at the R17 coat-protein binding site, which can form only three to four base pairs with the 3′-terminus of 16S RNA, is inhibited by the presence of factor *i* (refs 54–56), whereas initiation at the A-protein site (7 possible base pairs) is unaffected[55].

The ribosomal protein S_{12} is also clearly involved in specific initation on mRNA (ref. 12). However, its role may result from its ability to interact with initiation factors on the 30S subunit[12], perhaps potentiating the specific recognition by the 3′-end of

16S RNA. Using bifunctional cross-linking reagents it has recently been shown (Heimark, R., and Traut, R. T., and Bollen, A., Kahan, L., Cozzone, A., Hershey, J. W. B. and Traut, R. R., quoted in ref. 49.) that S_1 (factor *i*), S_{12} and IF-3 give cross-linked products in the 30S subunit, and are presumably close to the 3′-end of 16S RNA, since S_1 can be chemically linked to the 3′-terminal nucleoside[50].

We therefore propose that the cistron specificity of bacterial ribosomes previously ascribed to the 30S subunit, can be further localised to a pyrimidine-rich stretch of about ten nucleotides at the 3′-terminus of the 16S ribosomal RNA. We suggest that the degree of complementarity of this region with a purine-rich segment within the ribosome binding site sequence on bacterial mRNA determines the intrinsic capacity of the ribosome to translate a particular cistron. The finer controls imposed on initiation at different cistrons may involve both the accessibility of the purine-rich segment to the 3′-terminus as determined by the secondary structure of the initiation region, and the availability of certain initiation factors which would modify the proposed interaction.

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letters to nature

Submillimetre brightness spike at the solar limb

WE report here the first complete phase of the reduction of data obtained during the solar eclipse of June 30, 1973 from the high altitude moving platform provided by Concorde 001. Reports of the flight[1] and of an optical measurement of chromospheric thickness[2] are already published. Submillimetre observations were made at both the second and third contacts, using a rapid-scanning Michelson interferometer. At the second contact one complete interferogram of resolution 1 cm^{-1} was measured each second, but the limited passband of the detector, an InSb Rollin instrument[3], was found to have attenuated the highest frequencies (above 200 Hz) so these data have not been used. At the third contact 10 s were taken per interferogram. The instrument response was satisfactory, but the record needed subsequent digitisation, rendering analysis much lengthier, since an electronic analogue Fourier transformer had been built for 1 s interferograms, but was not usable for the 10 s data.

Figure 1 shows a plot of solar flux against the lunar limb position in 3 passbands, centred at 400 μm, 800 μm, and 1,200 μm. Radiation from the image of the revealed solar crescent, plus that from the lunar disk, and from the sky field was plotted against time in each band. Time was then correlated with the limb positions using aircraft coordinates and astronomical data. The lunar and sky contributions were then subtracted, leaving the solar contribution as a function of the distance of the lunar limb from the solar optical limb. Corrections for several effects were taken into account. First, for atmospheric emission and absorption, the latter averaging less than 10% in the shortest, most obscured waveband. Second, for spectrally selective absorption and emission by the 12 mm thick, crystal quartz, window of the aircraft. Third, for spectrally selective absorption by the TPX lenses in the spectrometer and the TPX detector window. Finally, for the wide lobes of the 12-cm telescope, which had 30′ diffraction limited resolution at 1,200 μm. The sky brightness temperature above 1,200 μm averaged 15 K above 57,000 feet, giving an accuracy limit of 1% of the total signal, which limited the relative radiometry.

The separate contributions from the whole lunar disk and from the sky were measured with considerable precision during the approximately 65 min of submillimetre totality, by integrating alternately on and off the image of the lunar disk. During the 7 min observing time starting at the origin of Fig. 1 the power on to the detector from the Moon, within the instrument passband, was 3×10^{-8} W; from the sky it averaged 1.5×10^{-8} W. The power from the solar crescent when the optical