Peptide-chain initiation in duodenal mucosa of rachitic chicks after $1\alpha,25$ -dihydroxycholecalciferol administration

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The post-ribosomal fraction of chick duodenal mucosa contains Met-tRNA^{Met}-binding protein(s) that behaves like the eukaryotic initiation factor (eIF-2) in protein synthesis. The binding activity of cytosol protein can be measured by retention of the radioactive complex formed on a nitrocellulose membrane. Complex-formation requires Met-tRNA^{Met} and GTP or guanosine [β,γ -methylene]triphosphate, and is inhibited by aurintricarboxylic acid. The ternary initiation complex thus formed can bind to ribosomal particles from chick intestine. By sucrose-density-gradient centrifugation, [^{35}S]Met-tRNA^{Met} was found to bind exclusively to 40S and not to 60S ribosomal subunit particles. In the duodenal mucosa of rachitic chicks the ability of the cytosol proteins to promote the binding of Met-tRNA^{Met} to ribosomal particles via ternary-complex formation is detectably increased by 3 h after injection of $1\alpha,25$ -dihydroxycholecalciferol, the active form of vitamin D. Cholecalciferol and ergocalciferol under the same experimental conditions failed to stimulate Met-tRNA^{Met}-binding activity.

$1\alpha,25$ -(OH) $_2$ D $_3$ has been reported to mediate the biochemical events that result in increased Ca $^{2+}$ absorption from the intestine (Kodicek, 1974). This seco-steroid, considered to be the hormonal form of vitamin D $_3$, has been shown to associate in chick duodenal mucosa with a specific cytoplasmic receptor (Brumbaugh & Haussler, 1974) and then to translocate to the chromatin fraction (Lawson & Wilson, 1974), where it alters transcriptional events (Zerwekh *et al.*, 1976), enhances RNA synthesis (Tsai & Norman, 1973) and leads to the stimulation of synthesis of several proteins (Lawson & Davie, 1979). Enhancement of specific enzyme activities and alteration of cellular functions by $1\alpha,25$ -(OH) $_2$ D $_3$ have been also reported (Wilson & Lawson, 1981; Mezzetti *et al.*, 1981; Shinki *et al.*, 1981).

So far, studies of the control of gene expression in chick intestinal cells have been mostly addressed to the effect exerted by the hormone at the transcriptional level, demonstrating that specific mRNA molecules are preferentially produced in

response to $1\alpha,25$ -(OH) $_2$ D $_3$ (Emtage *et al.*, 1974; Siebert *et al.*, 1982).

With regard to translation, a possible hormonal effect on the control of protein synthesis at the step of initiation has not been yet considered in this experimental system.

It has been established that in eukaryotic cells eIF-2 forms a ternary initiation complex with GTP and Met-tRNA^{Met} as the first step in peptide-chain initiation. The next step involves the transfer of Met-tRNA^{Met} to the 40S ribosomal subunit to form the pre-initiation complex (Hershey, 1982). Several examples of translational control of protein synthesis in eukaryotic cells are known to operate by regulating eIF-2-mediated Met-tRNA^{Met}-binding activity (Austin & Kay, 1982; Jackson, 1982). Assay of this binding activity is therefore extremely useful in examining the regulation of initiation of protein synthesis in either specialized or less-specialized eukaryotic cells (Liang *et al.*, 1977; Jagus *et al.*, 1981).

The present paper describes formation of the ternary initiation complex by post-ribosomal supernatant from chick duodenal mucosa and its binding to homologous ribosomal particles. In addition, evidence of a hormonal action exerted by $1\alpha,25$ -(OH) $_2$ D $_3$ on the initiation of polypeptide synthesis in this tissue is also given.

Abbreviations used: $1\alpha,25$ -(OH) $_2$ D $_3$, $1\alpha,25$ -dihydroxycholecalciferol; initiator tRNA or Met-tRNA^{Met}, initiator species of methionyl-tRNA^{Met}; eIF-2, eukaryotic initiation factor 2.

Materials and methods

Chemicals

1 α ,25-Dihydroxycholecalciferol was generously given by Prodotti Roche, Milano, Italy.

L-[³⁵S]Methionine (1165 Ci/mmol) was obtained from New England Nuclear G.m.b.H. (Dreieichen, West Germany); whole stripped tRNA was purchased from Grand Island Biological Co. (Grand Island, NY, U.S.A.); crude *Escherichia coli* aminoacyl-tRNA synthetase, cholecalciferol and ergocalciferol were the products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). Rat liver tRNA was acylated with [³⁵S]methionine by *Escherichia coli* synthetase, by a published procedure (Stanley, 1972). The methionylated tRNA was recovered by ethanol precipitation after phenol extraction. The precipitate was dissolved in and dialysed against 0.01 M-NaCl. The specific radioactivity obtained was 3.32 μ Ci/ A_{260} unit. All other reagents were of commercial origin, of the highest grade of purity.

Animals and tissue preparation

Animals used were 4–5-week-old Arbor Acres cockerels fed since hatching with a vitamin-D-deficient diet (McNutt & Haussler, 1973).

For the study of the 1 α ,25-(OH)₂D₃ effect, experiments *in vivo* were performed in duplicate and were repeated at least four times to ensure reliability. Vitamin D-dosed chicks received 150 ng of 1 α ,25-(OH)₂D₃ intracardially in 0.1 ml of ethanol/propylene glycol (1:9, v/v) before being killed at the times stated. The control animals received an injection of the same amount of vehicle.

All manipulations were performed in the cold (0–2°C). The duodenum was removed, rinsed in a medium containing 40 mM-Tris/HCl, pH 7.5, 25 mM-KCl and 3 mM-dithiothreitol, and mucosal layer was separated from the underlying muscle layers with a glass slide.

Isolation of subcellular fractions

Duodenal mucosa (4g) was homogenized in 4 vol. of the same buffer with a Potter-Elvehjem homogenizer and Teflon pestle. The homogenate was centrifuged in a Spinco SW60 rotor for 1 h at 2°C and 250 000g (r_{av} 9.2 cm). The upper three-quarters of the supernatant were taken and used fresh, as the cytosol fraction for the assays.

Ribosomal particles were prepared from duodenal mucosa of 3–4-week-old chicks raised on a normal diet. The tissue (10g) was homogenized with 4 vol. of 0.25 M-sucrose/TKMD (TKMD medium contained 40 mM-Tris/HCl, pH 7.5, 25 mM-KCl, 5 mM-MgCl₂ and 3 mM-dithiothreitol). Homogenization and centrifugation were performed as described above in the presence of

150 μ g of heparin/ml. The pellet was resuspended in 0.25 M-sucrose/TKMD containing 0.35% sodium deoxycholate (3 ml/g of tissue). A post-mitochondrial supernatant fraction (S20) was prepared by centrifuging the suspension for 15 min at 2°C and 20 000g. Portions of S20 fraction were layered over a 3 ml cushion of 2 M-sucrose/TKMD at the bottom. Centrifugation in a Spinco 50 rotor for 23 h at 2°C and 105 000g (r_{av} 5.4 cm) yielded a ribosomal pellet, which was rinsed and suspended in 0.25 M-sucrose/TKMD (80 A_{260} units/ml). The concentration of ribosomes was estimated by u.v.-absorption measurement, assuming 1 A_{260} unit = 80 μ g of ribosomes (A_{260}/A_{280} = 1.8).

Ribosomal subunit particles were prepared as described by Falvey & Staehelin (1970). The 40S and 60S subunits were separated by sucrose-gradient centrifugation, collected and sedimented by centrifugation overnight at 300 000g as reported by Schreier & Staehelin (1973). The purified subunits were resuspended in TKMD buffer (20 A_{260} units/ml) and stored at –80°C until used. Repeated freezing and thawing did not cause loss of activity.

Assay of initiation-factor binding of [³⁵S]Met-tRNA^{Met}

The assay was performed in a final volume of 0.3 ml of a reaction mixture containing 40 mM-Tris/HCl, pH 7.5, 25 mM-KCl, 3 mM-dithiothreitol and various amounts of MgCl₂, GTP and cytosol protein as indicated. The reaction was started by addition of 0.03 A_{260} unit of [³⁵S]Met-tRNA^{Met}, and the mixture was incubated at 30°C for 10 min. The reaction was stopped by the addition of 3 ml of ice-cold 20 mM-Tris/HCl (pH 7.5)/40 mM-KCl/5 mM-MgCl₂. The mixture was filtered through a Millipore membrane (type HAWP) and washed with 2 $\times$ 3 ml of the same buffer. The filters were dried and counted for radioactivity in a toluene-based scintillation 'cocktail' (Mezzetti *et al.*, 1980). At 30°C the formation of the ternary initiation complex under the standard conditions indicated reached a maximum within a few minutes, and it was too rapid for estimates of initial rates to be made. Values reported in the present paper represent the steady state of the reaction.

Assay of ribosomal binding of [³⁵S]Met-tRNA^{Met}

The assay of binding of initiator tRNA to ribosomal particles was performed in a final volume of 0.3 ml of a reaction mixture containing 40 mM-Tris/HCl, pH 7.5, 50 mM-KCl, 3 mM-dithiothreitol, 5 mM-MgCl₂, 2.5 mM-GTP, and various amounts of cytosol protein and ribosomal particles, as indicated. When purified 40S subunits were used in the binding assay, 5 μ g of (AUG) was added to the reaction mixture. The reaction was started by

addition of 0.03 A_{260} unit of [35 S]Met-tRNA $^{\text{Met}}$ and the mixture was incubated at 30°C for 15–30 min. The formation of initiator-tRNA-ribosomal-particle complexes could be analysed by the Millipore filtration technique described above, or by sucrose-density centrifugation after fixing of the complexes by the addition of 10 μ l of 25% (w/w) glutaraldehyde solution at pH 7. Fixed samples (200 μ l) were layered over 3.4 ml of linear 5–20% sucrose gradients in TKMD buffer. Gradients were centrifuged for 1 h at 2°C and 300 000 g in the SW60 Spinco rotor; 0.1 ml fractions were collected from the bottom of the gradients and their radioactivity was measured.

The concentration of proteins was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Binding of Met-tRNA $^{\text{Met}}$ to native ribosomal small subunits is the first step in the process of initiation of protein synthesis in eukaryotic cells (Jagus *et al.*, 1981; Jackson, 1982). This is mediated by the initiation factor eIF-2, which forms a ternary initiator complex with Met-tRNA $^{\text{Met}}$ and GTP and binds them to native small ribosomal subunits. Since the eIF-2-Met-tRNA $^{\text{Met}}$ -GTP complex is stable and binds to nitrocellulose filters, membrane filtration has been widely employed to assay ternary-complex formation. Conditions required for maximal formation and binding of the complex in our experimental system were determined. Fig. 1 shows that duodenal cytosol does contain component(s) able to bind radioactive initiator tRNA in the presence of GTP. This nucleotide strongly stimulates the binding reaction, which reaches a maximum value at a concentration of about 2 mM. If the amounts of GTP and [35 S]Met-tRNA $^{\text{Met}}$ were kept constant, the binding reaction was linearly proportional to increasing amounts of duodenal cytosol preparation added to the test tube until a plateau was reached. In the duodenal mucosa system, under the standard assay conditions used the non-specific GTP-independent binding reaction was usually low. Nevertheless, to avoid erroneous results, unless stated otherwise, all binding data reported in this paper have been corrected for the respective GTP-independent reaction.

The cytosol binding activity was apparently due to protein, since the activity could be abolished by heating the cytosol preparation at 80°C for 15 min, but it was not lost by precipitation with (NH $_4$) $_2$ SO $_4$ or after extensive dialysis.

As shown in Table 1, GDP, GMP and cyclic nucleotides such as cyclic AMP or cyclic GMP, did not support the binding reactions, whereas the

Fig. 1. Binding of Met-tRNA $^{\text{Met}}$ to duodenal cytosol proteins as a function of GTP

[35 S]Met-tRNA $^{\text{Met}}$ -binding activity of cytosol proteins from rachitic chicks was determined by the filtration assay as described in the Materials and methods section: $\circ$, without cytosol protein; $\bullet$, with 140 μ g of duodenal cytosol protein from rachitic chicks.

non-hydrolyzable GTP analogue guanosine [β , γ -methylene]triphosphate supported about 60% of full activity. Aurintricarboxylic acid at a concentration known to suppress the initiation process in other experimental systems (Lodish *et al.*, 1971) abolished the duodenal binding activity. The initiator tRNA could not be replaced by [35 S]methionine and deacylated tRNA in the binding to cytoplasmic factors. The inclusion in the reaction mixture of high amounts of uncharged rat liver tRNA did not alter the rate or extent of Met-tRNA $^{\text{Met}}$ binding (results not shown), indicating that tRNA $^{\text{Met}}$ as well as other uncharged species do not compete efficiently with initiator tRNA for binding to cytosol protein. It has been shown that in reticulocytes GDP can bind to eIF-2 and causes inhibition of ternary-complex formation (Walton & Gill, 1975). In such an experimental system, Benne *et al.* (1979) have reported the use of a GTP-generating system consisting of phosphocreatine, creatine kinase and ATP in Met-tRNA $^{\text{Met}}$ -binding assays. Table 1 also shows that the addition of a GTP-generating system to our binding assay stimulated by about 40% ternary-complex formation by cytosol protein. This is possibly due to

Table 1. Requirements for [35 S]Met-tRNA Met binding to cytosol proteins

The complete assay mixture contained 2mM-GTP and 200 μ g of cytosol protein prepared from rachitic chicks as well as other compounds described in the Materials and methods section. Where indicated, MgATP (1 mM), phosphocreatine (5 mM), creatine kinase (5 units), aurointricarboxylic acid (60 μ M), cyclic nucleotides, GDP, GMP and guanosine [β , γ -methylene]triphosphate (all 2 mM), [35 S]methionine (0.06 μ Ci; 0.1 μ M) and stripped rat liver tRNA (0.02 A_{260} unit) were present from the start of incubation. Other experimental details are described in the Materials and methods section. Results are means $\pm$ S.E.M. for four separate experiments.

Additions to, or omissions from, incubation mixture	[35 S]Met-tRNA Met retained by Millipore filter (d.p.m./filter)
Complete	4071 $\pm$ 302
+ ATP + phosphocreatine + creatine kinase	5772 $\pm$ 391
+ Aurintricarboxylic acid	118 $\pm$ 20
- GTP	874 $\pm$ 63
- GTP + cyclic AMP	902 $\pm$ 63
- GTP + cyclic GMP	803 $\pm$ 58
- GTP + GDP	808 $\pm$ 51
- GTP + GMP	918 $\pm$ 60
- GTP + guanosine [β , γ -methylene]-triphosphate	2496 $\pm$ 198
- [35 S]Met-tRNA Met + [35 S]methionine + tRNA	101 $\pm$ 20

removal of inhibitory concentration of GDP present in duodenal preparations or formed by GTP-ases. For this reason, and in order to have a GTP/ATP ratio maintained at a standard value in all protein samples, we routinely included the GTP-generating system in our assay mixture.

Other experiments have shown that the salt concentrations used in the standard binding assay were optimal and that in the presence of the GTP-generating system Mg^{2+} ions added to the binding assay stimulated the GTP-dependent ternary-complex formation at concentrations of 1–5 mM. Furthermore, inclusion of Mg^{2+} in the buffer used to wash the nitrocellulose filters after binding of the ternary complex appeared to decrease non-specific binding significantly.

Fig. 2 shows the effect of a single intracardial injection of 150 ng of various vitamin D metabolites such as cholecalciferol, ergocalciferol and $1\alpha,25-(OH)_2D_3$ on the ability of duodenal cytosol protein to bind Met-tRNA Met in the presence of GTP. In this experiment initiator-tRNA-binding activity was measured 3 h after dosing with each metabolite or vehicle, because this time period appeared most suitable to detect early stimulation of Met-tRNA Met -

Fig. 2. Effect of a single intracardial dose of vitamin D metabolites on Met-tRNA Met -binding activity of duodenal mucosa cytosol proteins

Rachitic chicks were injected with cholecalciferol (■), ergocalciferol (▲), $1\alpha,25-(OH)_2D_3$ (●) or vehicle (○) and killed 3 h later. Various amounts of cytosol protein were assayed for their ability to bind [35 S]Met-tRNA Met by the filtration assay. The complete assay mixture contained 2mM-GTP, 1mM-ATP, 5mM-MgCl $_2$, 5mM-phosphocreatine and 5 units of creatine kinase as well as other components as described in the Materials and methods section.

binding activity and to minimize further hydroxylations of the metabolites. Among the compounds tested, only $1\alpha,25-(OH)_2D_3$ was found to induce a significant increase in the ability of duodenal cytosol protein to form a ternary initiation complex. The selective effect was clearly observed with various protein concentrations in the assay, and apparently was not due to a limitation in the components needed for the binding activity, nor to RNA or different concentrations of ions in the cytosol preparation.

Increased binding activity in cytosol from $1\alpha,25-(OH)_2D_3$ -dosed chicks did not appear to result from different dilution of [35 S]Met-tRNA Met by endogenous substrate or from differential degradation. Ternary-initiator-complex formation was investigated over a wide range of [35 S]Met-tRNA Met concentration (Fig. 3). The increased activity in cytosol from $1\alpha,25-(OH)_2D_3$ -dosed chicks was apparent at all initiator-tRNA concentrations tested. Addition of cytosol fractions from hormone- or vehicle-injected animals to the assay

Fig. 3. Effect of availability of Met-tRNA^{Met} on binding activity of duodenal cytosol proteins from rachitic and 1 α ,25-(OH)₂D₃-treated chicks

Rachitic chicks were injected with 1 α ,25-(OH)₂D₃ (●) or with vehicle (○) and killed 3 h later. Cytosol (80 μ g of protein) was assayed for its ability to bind various amounts of [³⁵S]Met-tRNA^{Met} by the filtration method. The complete assay mixture contained 2 mM-GTP, 1 mM-ATP, 5 mM-MgCl₂, 5 mM-phosphocreatine, 5 units of creatine kinase as well as other components as described in the Materials and methods section.

system slightly decreased the amount of [³⁵S]Met-tRNA^{Met} that remained acylated during incubation (by 15–20%); however, the rate of deacylation, measured by a DEAE-Sephadex A-25 gel-chromatography method (Mezzetti, 1981), was similar for each tissue source (results not shown).

In order to confirm the finding that initiator-tRNA-binding activity to cytosolic proteins was increased after a single dose of 1 α ,25-(OH)₂D₃, we have undertaken experiments involving binding of [³⁵S]Met-tRNA^{Met} to ribosomal particles. Whole ribosome preparations contained polyribosomes, monoribosomes and native subunits as well, and were largely free of small molecules such as GTP and ATP. As with other eukaryotic systems (Ochoa & Mazumder, 1974), we found that also with chick duodenal mucosa the retention of [³⁵S]-Met-tRNA^{Met} by the nitrocellulose filter was dependent on the presence in the reaction mixture of ribosomal preparation, GTP and cytosol pro-

Table 2. Requirements for binding of [³⁵S]Met-tRNA^{Met} to duodenal mucosa ribosomal particles

Ribosomal particles were prepared from chicks raised on a normal diet. Cytosol protein was prepared from rachitic chicks. The complete assay mixture contained 40 μ g of cytosol protein and 2.5 A₂₆₀ units of ribosomal particles, as well as other components described in the Materials and methods section. Where indicated, ATP (1 mM), phosphocreatine (5 mM), creatine kinase (5 units), cyclic nucleotides, GDP or GMP (all 2.5 mM) were present from the start of incubation. The incubation was performed for 15 min at 30°C and the binding activity was assayed by the Millipore filtration method. Results are means $\pm$ S.E.M. for four separate determinations.

Additions to, or omissions from, incubation mixture	[³⁵ S]Met-tRNA ^{Met} retained by Millipore filter (d.p.m./filter)
Complete	3056 $\pm$ 287
+ ATP + phosphocreatine + creatine kinase	4238 $\pm$ 351
– GTP	421 $\pm$ 37
– GTP + cyclic AMP	383 $\pm$ 30
– GTP + cyclic GMP	442 $\pm$ 31
– GTP + GDP	360 $\pm$ 29
– GTP + GMP	394 $\pm$ 34
– ribosomal particles	201 $\pm$ 25
– cytosol	597 $\pm$ 50
– cytosol – ribosomal particles	87 $\pm$ 11

teins as a source of initiation factors (Table 2). Under standard assay conditions the maximal binding was obtained with 1.5–2.5 mM-GTP. As shown in Table 2, GDP, GMP or cyclic nucleotides could not replace GTP in supporting the binding reaction, indicating that the binding is mediated by initiation factors. Addition of a GTP-generating system in the reaction mixture resulted, as in the ternary-complex-formation assay, in about 40% stimulation of binding activity.

As shown in Fig. 4, when purified ribosomal subunit particles were used in the assay, the 40S (panel a) but not the 60S fraction (panel b) was found to be active. The binding of Met-tRNA^{Met} to the 40S ribosomal subunit was absolutely dependent on the presence of the cytosol protein factors and GTP, whereas GDP and other nucleotides did not support the binding reaction. Thus it is apparent that in the chick intestine system the formation of the Met-tRNA^{Met}-eIF-2-GTP ternary complex and its binding to the ribosome subunit occur in the same way as in other eukaryotic systems.

As shown in Fig. 5, after 3 h from a single intracardial injection of 1 α ,25-(OH)₂D₃ the ability of cytosol factors to support the initiation of protein synthesis by ribosomal particles was remarkably

Fig. 4. Formation of 40S preinitiation complex analysed by sucrose-density-gradient centrifugation. Cytosol (350 μ g of protein) from duodenal mucosa of rachitic chicks (●) was studied for its ability to facilitate [35 S]Met-tRNA^{Met} binding to 0.6 A_{260} unit of 40S (a) or to 1.2 A_{260} unit of 60S (b) ribosomal subunits. The incubation was carried out for 30 min at 30°C. Other conditions as well as assay components and sucrose-density-gradient centrifugation were as described in the Materials and methods section. The arrows indicate the positions to which purified 40S or 60S subunits sedimented in parallel control gradients.

increased above the value shown by vehicle-injected rachitic chicks. The stimulatory effect was apparent at various protein concentrations, as previously shown for ternary-complex formation. We have consistently observed increases (20–80%) in most of the experiments so far performed. Only two groups out of more than 40 did not respond to $1\alpha,25\text{-(OH)}_2\text{D}_3$ administration, and two gave an increase below 20%. When the cytosol proteins from vehicle-injected rachitic chicks and $1\alpha,25\text{-(OH)}_2\text{D}_3$ -dosed chicks were mixed in equal amounts, the resulting activity was nearly additive, suggesting that the increase in Met-tRNA^{Met}-binding activity observed is not due to the appearance or disappearance of a modulator in the cytosol preparations. No significant difference in the pattern of hormone stimulation of the binding activity was observed when purified 40S ribosomal subunits were used in the assay instead of whole ribosomes.

Discussion

The present work suggests that cytosol from chick duodenal mucosa contains a protein(s) that can interact with GTP and Met-tRNA^{Met} to form a soluble ternary complex that can be retained by nitrocellulose membranes. This complex then binds to the 40S (but not to the 60S) ribosomal subunit to form a 40S preinitiation complex in a way

similar to that proposed for other eukaryotic systems.

Moreover, data presented here suggest that the two reactions of the peptide-chain-initiation process that we have studied are sensitive to the active form of vitamin D. The effect of the hormone appears to be quite rapid, since we have consistently found remarkable stimulation in both Met-tRNA^{Met}-binding assays within 2–3 h after dosing the chicks.

Change in the rate of peptide-chain initiation is considered a rapid short-term control mechanism for modifying gene expression by changing the relative efficiency with which different mRNA species are translated (Bergmann & Lodish, 1979). However, when a cell is responding to a stimulus, initial alterations in the rate of protein synthesis can be achieved by changes in the rate of initiation (Liang *et al.*, 1977; Jagus *et al.*, 1981). Siebert *et al.* (1982) have reported that detectable polyribosomal Ca^{2+} -binding-protein mRNA activity is induced in duodenal mucosa of rachitic chicks 3 h after $1\alpha,25\text{-(OH)}_2\text{D}_3$ administration. This fact may correlate with the increased ability of cytosol proteins to support the initiation of protein synthesis found at approximately the same time. The two phenomena may represent different aspects of the same rapid response to hormone stimulation. In fact it is plausible that $1\alpha,25\text{-(OH)}_2\text{D}_3$ may activate

Fig. 5. Effect of 1 α ,25-(OH) $_2$ D $_3$ on the cytosol factors facilitating ribosomal binding of [35 S]Met-tRNA $_f^{\text{Met}}$. Rachitic chicks were injected with 1 α ,25-(OH) $_2$ D $_3$ (D) or vehicle (R) and killed 3 h later. The complete assay mixture contained 2.5 A $_{260}$ units of ribosomal particles, 2 mM-GTP, 1 mM-ATP, 5 mM-phosphocreatine, 5 units of creatine kinase as well as other components as described in the Materials and methods section. The incubation was carried out for 15 min at 30°C and the binding activity at various cytosol protein concentration was assayed by the filtration method. ○, Cytosol protein from R chicks; ●, cytosol protein from D chicks; ▲, cytosol proteins consisting of half R-cytosol and half D-cytosol protein ('R+D'). The radioactivity (521 d.p.m.) found in the absence of added cytosol fraction was deducted from the data.

ate factors that have a role in the transcription, translation and other processes, to ensure a well-coordinated and efficient regulation of gene expression in the duodenal cell.

Apparently binding of Met-tRNA $_f^{\text{Met}}$ to ribosomal particles is more sensitive than ternary-complex formation to the stimulatory effect of 1 α ,25-(OH) $_2$ D $_3$ (cf. Figs 2 and 5). In fact, using the same protein preparations from both vehicle-injected rachitic chicks and hormone-dosed chicks we found a 20–25% stimulatory effect when ribosomal particles or 40S subunits were present in the assay. This fact suggests that possibly more than one control factor may be involved in stimulation of Met-tRNA $_f^{\text{Met}}$ -binding activity to ribosomal particles by 1 α ,25-(OH) $_2$ D $_3$.

Peptide-chain initiation in erythroid and non-

erythroid cells has been shown to be mediated by several factors and cofactors (Leader *et al.*, 1972; Picciano *et al.*, 1973; Austin & Clemens, 1980; Jagus *et al.*, 1981). Whether 1 α ,25-(OH) $_2$ D $_3$ in the intestinal cell affects the activity of these known proteins or others not yet characterized requires further investigation.

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