In mammalian cells valyl-tRNA synthetase (ValRS) forms a high $M_c$ complex with the four subunits of elongation factor EF-1H. The $\beta$, $\gamma$, and $\delta$ subunits, that contribute the guanine nucleotide exchange activity of EF-1H, are tightly associated with the NH$_2$-terminal polypeptide extension of valyl-tRNA synthetase. In this study, we have examined the possibility that the functioning of the companion enzyme EF-1$\alpha$ could regulate valyl-tRNA synthetase activity. We show here that the addition of EF-1$\alpha$ and GTP in excess in the aminoacylation mixture is accompanied by a 2-fold stimulation of valyl-tRNA$^{\text{val}}$ synthesis catalyzed by the valyl-tRNA synthetase component of the ValRS-EF-1H complex. This effect is not observed in the presence of EF-1$\alpha$ and GDP or EF-Tu-GDP and requires association of valyl-tRNA synthetase within the ValRS-EF-1H complex. Since valyl-tRNA synthetase and elongation factor EF-1$\alpha$ catalyze two consecutive steps of the in vivo tRNA cycle, aminoacylation and formation of the ternary complex EF-1$\alpha$-GTP-Val-tRNA$^{\text{val}}$ that serves as a vector of tRNA from the synthetase to the ribosome, the data suggest a coordinate regulation of these two successive reactions. The EF-1$\alpha$-GTP-dependent stimulation of valyl-tRNA synthetase synthetase activity provides further evidence for tRNA channeling during protein synthesis in mammalian cells.

Aminoacyl-tRNA is the donor of amino acid in ribosomal protein synthesis. The tRNA molecule is aminoacylated with the corresponding amino acid by an aminoacyl-tRNA synthetase synthetase, the aminoacyl-tRNA is converted to a ternary complex with elongation factor 1$\alpha$, to give the immediate precursor of amino acid for protein synthesis: EF-1$\alpha$-GTP-aminoacyl-tRNA. Several lines of evidence have suggested that in mammalian cells the translational apparatus is highly organized. In particular, association of the protein vectors of tRNA, aminoacyl-tRNA synthetases and elongation factors, with the cytoskeletal framework has been reported (1–3) and colocalization of these components described (4). The isolation and characterization of supramolecular assemblies of aminoacyl-tRNA synthetases and elongation factors (5–7) have provided structural evidence for the subcellular organization of the protein synthesis machinery. The existence of a channeled tRNA cycle during mammalian protein synthesis provides functional evidence for cellular compartmentalization of translation (8–10). According to the proposed channeling scheme, aminoacyl-tRNAs are vectorially transferred from the aminoacyl-tRNA synthetases to the ribosomes as ternary complexes EF-1$\alpha$-GTP-aminoacyl-tRNA (8, 10). Moreover, the GDP form of EF-1$\alpha$ could be involved in the capture of deacylated tRNA at the exit site of the ribosome and its delivery to the synthetase (11).

Channeling, or direct transfer of metabolites from one enzyme to another in a metabolic pathway, is believed to increase significantly the efficiency of the overall reaction (12). For sequential metabolic enzymes, the stimulation of activity of the first enzyme induced by a protein-protein interaction with the second enzyme provides a structural basis for a channeling mechanism. As far as the protein biosynthesis machinery is concerned, the possible regulation of aspartyl- (13) and phenylalanyl- (14) tRNA synthetase activities by elongation factor EF-1$\alpha$ has been reported. In both cases, no stable protein-protein interaction between the synthetase and the elongation factor was detected. In connection with tRNA channeling from aminoacyl-tRNA synthetase to EF-1$\alpha$, two enzymes ensuring consecutive steps of the tRNA cycle, the multienzyme complex containing valyl-tRNA synthetase and EF-1H deserves special mention.

The only stable macromolecular assemblage that involves an aminoacyl-tRNA synthetase synthetase and elongation factor 1$\alpha$ is the ValRS$^\text{Val}$-EF-1H complex (15, 16). EF-1H, the “heavy” form of the translation elongation factor 1, is a pentameric complex of the four subunits $\alpha$, $\beta$, $\gamma$, and $\delta$ in molar ratio 2:1:1:1 (17). EF-1$\alpha$ forms a ternary complex with aminoacyl-tRNA and GTP to give the active species of amino acid for protein synthesis and delivers aminoacyl-tRNA to the A site of the ribosome. The EF-1$\beta\gamma\delta$ subunits contribute the guanine nucleotide exchange activity to regenerate EF-1$\alpha$-GTP from EF-1$\alpha$-GDP. The finding that in mammalian cells valyl-tRNA synthetase is exclusively found as a complex with EF-1H has suggested that this association might contribute an essential function in vivo. Here we show that EF-1$\alpha$ controls the aminoacylation reaction catalyzed by ValRS. Our results demonstrate that association of ValRS with EF-1H is absolutely required for the stimulation of activity of the first enzyme induced by a protein-protein interaction with the second enzyme provides a structural basis for a channeling mechanism.

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# The abbreviations used are: ValRS, valyl-tRNA synthetase; DTE, 1,4-dithioerythritol; GMP-PNP, $\beta$-imidoguanosine 5‘-triphosphate.
EF-1{alpha} Regulates Valyl-tRNA Synthetase Activity

**EXPERIMENTAL PROCEDURES**

**Protein Purification—**The ValRS-EF-1H complex was isolated from rabbit liver according to a procedure adapted from that used to isolate the multisynthetase complex (18). Briefly, livers (1 kg) from 13 rabbits were homogenized in three portions (w/v; total volume 2 liters) of extraction buffer (50 mM Tris·HCl (pH 7.5), 5 mM MgCl₂, 0.1 mM EDTA, 10% glycerol, 1 mM DTE (1,4-dithiothreitol)) containing 1 mM disopropyl fluorophosphate. Postmitochondrial supernatant was adjusted to 2% polyethylene glycol-6000 by addition of a 50% stock solution in extraction buffer and stirred for 30 min at 4 °C. After centrifugation at 10,000 × g for 30 min, the supernatant was recovered, adjusted to 5% polyethylene glycol-6000, and stirred for 30 min at 4 °C. After centrifugation, the precipitate was dissolved in 200 ml of 25 mM potassium phosphate (pH 7.6), 5% glycerol, and 10 mM 2-mercaptoethanol. The homogeneous solution was applied to a Bio-Gel A-5m column (Bio-Rad; 90 × 910 mm) equilibrated with the same buffer containing 10% glycerol. Fractions containing valyl-tRNA synthetase activity were combined and loaded on a tRNA-Sepharose column (50 mm × 90 mm) equilibrated with the same buffer. The enzyme was eluted by a linear gradient in the same buffer. Fractions with ValRS activity were pooled, diluted by addition of 800 μl of ice-cold exchange buffer (20 mM Tris·HCl (pH 7.5), 10 mM magnesium acetate, 50 mM NH₄Cl, 0.1 mg/ml bovine serum albumin). The exchange reaction was conducted at 0 °C after addition of exchange buffer containing nucleotide and specified exchange factors. Aliquots were taken at times indicated and immediately solubilized through nitrocellulose filters (Sartorius; pore size 0.45 μm). Filters were washed three times with 1 ml of ice-cold washing buffer (20 mM Tris·HCl (pH 7.5), 10 mM MgCl₂, 100 mM NH₄Cl, 0.1 mg/ml bovine serum albumin), dried, and counted in Lipoluma scintillation fluid.

To obtain the GTP form of bacterial EF-Tu, purified EF-Tu was incubated with 100 μM GTP in an incubation mixture containing 25 mM Tris·HCl (pH 7.5), 50 mM NH₄Cl, 10 mM MgCl₂, 1 mM DTE, 0.5 mM EDTA, in the presence of pyruvate kinase (5 μg/ml) and phosphoenolpyruvate (1 mM) to remove traces of GDP. Incubation was conducted at 30 °C for 15 min, and the EF-Tu·GTP preparation was used immediately.

**RESULTS**

**EF-1{alpha}GTP Stimulates ValRS Activity in the ValRS-EF-1H Complex—**Earlier studies suggested that tRNA aminoacylation catalyzed by ValRS is independent of its association with EF-1{alpha}. Indeed, the intrinsic specific activity of the enzyme, determined in the aminoacylation reaction, was not affected following its dissociation from the EF-1H complex (15, 19). However, whereas the EF-1β, γ, and δ subunits are tightly bound to ValRS, the EF-1{alpha} subunit can be easily depleted from the ValRS-EF-1H complex (21). This finding suggested that the ternary complex EF-1{alpha}GTP·Val-tRNAVal is readily dissociated from the ValRS-EF-1H complex following completion of a single aminoacylation cycle. We reasoned that if valylation of tRNAVal by ValRS is controlled by EF-1{alpha}, this effect should be detected only in the presence of an excess of free EF-1{alpha}. If ValRS and EF-1{alpha} activities are coupled, as expected if tRNA is channeled from the synthetase to the elongation factor, addition in the reaction mixture of free EF-1{alpha} in excess, that could be effectively transformed into its EF-1{alpha}GTP species by the β, γ, and δ subunits of EF-1H in the presence of GTP, should result in an enhanced rate of Val-tRNAVal formation. When all EF-1{alpha} is transformed to a ternary complex with the aminoacylated tRNA, this stimulation should cease.

Therefore, we devised an aminoacylation assay designated to test a putative effect of EF-1{alpha}GTP on the valylation efficiency catalyzed by the ValRS component of the ValRS-EF-1H complex. In the assay procedure used in our study, the reaction mixture containing the ValRS-EF-1H complex and free EF-1{alpha} in excess, but deprived of tRNA, was incubated 2 min at 25 °C before the aminoacylation reaction was started by addition of the tRNA substrate. This preincubation was necessary to avoid a lag in the time course experiments described below. Presumably, EF-1H is preloaded with EF-1{alpha}GTP during this initial incubation. When catalytic amounts of the ValRS-EF-1H complex (1.5 nM) is incubated in the presence of an excess of free EF-1{alpha} (500 nM) and saturating amounts of the substrates for the aminoacylation reaction, in the presence of GTP to produce the active species EF-1{alpha}GTP, the rate of valyl-tRNAVal synthesis was 2-fold increased, raising from 1.2 pmol/min to 2.1 pmol/min (Fig. 1A). GTP alone had no effect on the rate of Val-tRNAVal formation. This increased aminoacylation rate was especially observed during the first minutes of the incubation, when the free form of EF-1{alpha} is still in excess. As exemplified in Fig. 1B with a higher amount of the ValRS-EF-1H complex (3 nM), when consumption of free EF-1{alpha} is faster, the time course of valyl-tRNAVal formation is clearly biphasic. The initial rate in the presence of EF-1{alpha}GTP (4.7 pmol of valyl-tRNAVal formed/min) is 2-fold that observed in the absence of EF-1{alpha} (2.4 pmol/min). After the reaction proceeded for about 9 min, producing 45 pmol of valyl-tRNAVal, corresponding approximately to the amount of EF-1{alpha} added in the incubation mixture (50 pmol), the time course of aminoacylation returned...
EF-1α Regulates Valyl-tRNA Synthetase Activity

**EF-1α Stimulation of ValRS Activity Is GTP-dependent**—The GTP form of elongation factor EF-1α, and therefore its ability to contribute a ternary complex EF-1α\(_{GTP}\)Val-tRNA\(_{Val}\), is absolutely required to stimulate the aminoacylation activity of ValRS. Indeed, in the presence of GDP instead of GTP, the rate of valylation of tRNA\(_{Val}\) by the ValRS\(_{EF-1H}\) complex is not affected by the presence of a large excess (200-fold) of free EF-1α (Fig. 2). The nonhydrolyzable GTP analogue GMP-PNP did produce a stimulation of ValRS activity in the presence of EF-1α, albeit to a lesser extent, as compared with GTP (Fig. 2). This lower efficiency could be due to a slower rate of GDP/GMP-PNP exchange, as compared with the GDP/GTP exchange, leading to a decreased rate of dissociation of EF-1α\(_{GMP-PNP}\)Val-tRNA\(_{Val}\) from the ValRS\(_{EF-1H}\) complex, as compared with the regular ternary complex EF-1α\(_{GTP}\)Val-tRNA\(_{Val}\). Accordingly, the rate of dissociation of GDP from the EF-1α\(_{GDP}\) complex in the presence of an excess of free GMP-PNP is not as fast as that observed in the presence of GTP (Fig. 3). Similarly, EF-1α\(_{GMP-PCP}\) has a stronger affinity for the EF-1βγ subunits than EF-1α\(_{GTP}\), thus slowing down the exchange of GDP from EF-1α-GDP (22). Alternatively, the inability of GMP-PNP to mimic GTP for the stimulation of ValRS by EF-1α also suggests that GMP-PNP, from which P\(_i\) cannot be released, has not the potential of GTP to produce a conformational change in the ValRS\(_{EF-1H}\) complex that would favor the dissociation of the ternary complex EF-1α\(_{GTP}\)Val-tRNA\(_{Val}\).

**Bacterial EF-Tu Cannot Replace Eukaryotic EF-1α**—The simplest mechanism that might account for the observed EF-1α\(_{GTP}\)-dependent stimulation of ValRS activity would merely imply that the Val-tRNA\(_{Val}\) formed is sequestered by EF-1α\(_{GTP}\), thereby preventing product inhibition of ValRS activity. We tested this possibility by adding to the aminoacylation mixture the prokaryotic analogue of EF-1α, EF-Tu, that is known to interact, in its GTP-bound form, with eukaryotic aminoacyl-tRNAs (23). Since EF-Tu has a much lower affinity for GTP than for GDP to its control value determined in the absence of EF-1α. As shown in the inset of Fig. 1A, in the presence of GTP the duration of the stimulation and the apparent initial rate of valyl-tRNA\(_{Val}\) synthesis are functions of the amount of free EF-1α added in the incubation mixture.

**Fig. 1. Stimulation of [\(^{14}\)C]valyl-tRNA formation by valyl-tRNA synthetase in the presence of an excess of free EF-1α.** The time course of the tRNA\(_{Val}\) aminoacylation reaction catalyzed by ValRS from the purified ValRS:EF-1H complex was determined under the standard assay conditions, in 0.1 ml of the incubation mixture without additives (○) or containing 100 \(\mu\)M GTP (●) or 100 \(\mu\)M GTP and 500 nM free homogenous EF-1α (●). The mean value of three independent experiments and the associated S.E. values are indicated. A, the time course of Val-tRNA\(_{Val}\) formation by 1.5 nM ValRS. A possible biphasic fitting of the experimental values is indicated by a dotted line. Inset, dependence of the initial rates of Val-tRNA\(_{Val}\) formation on EF-1α concentration in the assay mixture, in the presence of 100 \(\mu\)M GTP. B, the time course of Val-tRNA\(_{Val}\) synthesis catalyzed by 3 nM ValRS.
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EF-1α from the ValRS-EF-1H complex is contributed by a protein-interaction requiring cognate factors from higher eukaryotic origin.

\[ \text{EF-1α + GDP} \]

\[ \text{EF-1α + GTP} \]

\[ \text{EF-1α + GMP-PNP} \]

\[ \text{+ GMP-PNP} \]

**Fig. 2.** Nucleotide-dependent activation of valyl-tRNA synthetase in the presence of EF-1α. The time course of valyl-tRNA\(^{\text{Val}}\) synthesis by ValRS (2.5 nM) from the purified ValRS-EF-1H complex was determined under the standard assay conditions, in 0.1 ml of the incubation mixture supplemented with 140 \(\mu\)M GMP-PNP (●) or containing free EF-1α in excess (500 nM) and 100 \(\mu\)M GDP (○), 140 \(\mu\)M GMP-PNP (△) or 100 \(\mu\)M GTP (■).

**Fig. 3.** Effect of nucleotide on the GDP exchange reaction catalyzed by EF-1H from the ValRS-EF-1H complex. The time course of GDP exchange from EF-1α\(^{[3]}\text{H}\text{GDP} \) (400 nM) was determined at 0 °C in the absence (●), or in the presence (○, □, △) of the EF-1H subunits (4 nM) from the ValRS-EF-1H complex. The reaction was initiated by addition of 100 \(\mu\)M GDP (●, □), 100 \(\mu\)M GTP (○), or 140 \(\mu\)M GMP-PNP (△), as described under “Experimental Procedures.” Radioactivity of \(^{[3]}\text{H}\text{GDP} \) bound to EF-1α before addition of a chasing nucleotide was taken as 100%.

(24) and cannot be charged with GTP by the nucleotide exchange subunits of EF-1H (unpublished observation), nucleotide exchange was performed by preincubation of the factor with an excess of GTP in the presence of phosphoenolpyruvate and pyruvate kinase. When EF-1α-GTP was substituted by EF-Tu-GTP in the aminoacylation reaction, no stimulatory effect of EF-Tu-GTP on the ValRS aminoacylation activity was observed at different concentrations of the factor (0.5–2 \(\mu\)M) (Fig. 4). This result suggested that the EF-1α-induced stimulation of Val-tRNA synthesis catalyzed by the ValRS component of the ValRS-EF-1H complex is contributed by a protein interaction requiring cognate factors from higher eukaryotic origin.

**EF-1αGTP Stimulation Requires a Native ValRS-EF-1H Complex—** To test for the requirement of the native ValRS-EF-1H assembly in the EF-1α-GTP stimulation of ValRS activity, two ValRS derivatives that behave as free species were isolated from the complex. It was shown previously that the ability of ValRS to associate with EF-1H is lost upon conversion by elastase of the native enzyme of 140 kDa to a fully active truncated form of 125 kDa (20). The 200-aa extension of ValRS lost upon elastase treatment is involved in the assembly of the ValRS-EF-1H complex through protein-protein interaction with the δ subunit of EF-1H. Furthermore, native ValRS can be dissociated from the EF-1H components in the presence of 0.5 M NaSCN, a chaotropic salt (19). The resulting monomeric ValRS species is isolated without loss of its aminoacylation activity.

These two monomeric ValRS species were assayed for their potential to be stimulated by EF-1α-GTP in conditions where the native ValRS-EF-1H species does. Neither the truncated free ValRS, nor the native free ValRS, could be activated by the addition of preformed EF-1α-GTP in the incubation mixture (Fig. 5). Further addition of the EF-1βγ subunits in the assay had no effect on ValRS activity (Fig. 5). As shown previously, the EF-1β and -γ subunits form a stable complex that efficiently exchanges the nucleotide from EF-1α-GDP but does not associate to the isolated 140- or 125-kDa ValRS species (20). In the absence of the EF-1β subunit the ValRS-EF-1H complex cannot be reconstituted. On the other hand, the EF-1β subunit associates with the native 140-kDa ValRS form (20), but in the absence of the β and γ subunits, this nucleotide exchange factor alone did not confer on EF-1α the ability to stimulate ValRS activity (not shown). Therefore, the association of ValRS with the β, γ, and δ subunits of EF-1H seems to be absolutely required to produce a functional interaction between the synthetase and elongation factor EF-1α. This result suggests that a proper positioning of EF-1α-GTP and ValRS-Val-tRNA\(^{\text{Val}}\) contributes an essential step for the activation of the synthetase activity. The presence in solution of a competitor protein (EF-1α-GTP or EF-Tu-GTP) with a high affinity for the newly synthesized aminoacyl-tRNA cannot per se explain the observed stimulation effect.

In the case of yeast ValRS, for which dissociation of the aminoacyl-tRNA was shown to be the rate-limiting step in the aminoacylation reaction (25), addition of EF-1α, EF-1βγ, and GTP proved to have no stimulatory effect (result not shown). Similarly, incubation of the multisynthetase complex from rab-
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**FIG. 4.** Effect of bacterial EF-Tu on eukaryotic valyl-tRNA synthetase activity. The time course of the aminoacylation reaction catalyzed by ValRS (1.7 nm) from the ValRS-EF-1H complex was followed in the presence of 100 μM GTP (□) or 100 μM GDP and 0.5 μM (△) or 2 μM (○) EF-Tu-GTP in the aminoacylation reaction. GTP-bound EF-Tu was obtained as described under “Experimental Procedures.”

**FIG. 5.** Effect of EF-1α on the activity of valyl-tRNA synthetase dissociated from the ValRS-EF-1H complex. The aminoacylation activity of the monomeric ValRS dissociated from the complex as a NH₂-terminally truncated form after elastase treatment (ValRS-ΔN) or recovered in the native state after NaSCN treatment of the complex (free ValRS) was assayed in the presence (□) or in the absence (○) of 100 μM GTP and 110 nM EF-1βγ were also added in the aminoacylation mixtures to convert EF-1α to its GTP-bound form. Enzyme concentrations were 3 nm for the two ValRS species and 500 nm for EF-1α.

EF-1α-mediated stimulation of ValRS can be detected in the presence of GDP but not of GTP. This finding clearly demonstrates the importance of the ternary complex EF-1αGTP-Val-tRNA Val in the stimulation mechanism. As expected, activation is observed as long as some free EF-1αGTP is available in the incubation mixture. When all EF-1α is converted to EF-1αGDP-Val-tRNA Val, ValRS is no longer stimulated, suggesting that EF-1α does not rapidly dissociate from GTP and Val-tRNA Val. Although the intrinsic GTPase activity of EF-1α is stimulated by the aminoacyl-tRNA (26), EF-1α does not appear to be efficiently recycled in this assay. Accordingly, it is possible to substitute GTP by GMP-PNP. This nonhydrolyzable GTP analogue can be regarded as mimicking the nucleotide substrate in its ground state complex. Therefore, the endogenous GTPase activity of EF-1α is not involved in the stimulation of ValRS activity.

Mammalian ValRS is a 1265-amino acid protein (27). As compared with its bacterial counterpart, the human enzyme displays a large NH₂-terminal polypeptide extension dispensable for its activity (20). In vitro reconstitution experiments have shown that this domain interacts with the δ subunit of EF-1H (20). The finding that neither the native enzyme of 140 kDa nor the truncated 125-kDa ValRS species are susceptible to EF-1α mediated stimulation also demonstrates that the NH₂-terminal extension of ValRS is not per se engaged in a functional interaction with EF-1α. Noteworthy, the NH₂-terminal polypeptide extension of the multifunctional glutamyl-prolyl-tRNA synthetase, and the p18 auxiliary component of the multisynthetase complex display sequence similarities with this domain (28). This observation led to the suggestion that these polypeptide domains could be involved in the transient anchoring of EF-1α to the complex. Though we were unable to detect any effect of EF-1αGTP on the activity of three components of the multisynthetase complex, namely lysyl-, aspartyl-, and methionyl-tRNA synthetases, we cannot rule out the possibility that a specialized adaptor molecule could be involved to provide a functional interaction between EF-1α and these synthetases, a role played by the EF-1βγδ subunits in the case of the ValRS-EF-1H complex.

The EF-1α-induced activation of tRNA Val aminoacylation by ValRS requires the whole ValRS-EF-1H complex to occur. Neither bacterial EF-Tu can substitute for mammalian EF-1α, nor free dissociated ValRS can mimic the ValRS component of the ValRS-EF-1H complex. Furthermore, the isolated guanine-
nucleotide exchange factors, EF-1γ or EF-1βγ, cannot individually sustain the activation of ValRS by EF-1α. All these results illustrate the direct connection between regulation of ValRS activity and the adequate association of ValRS and EF-1 subunits is within a macromolecular assemblage of defined composition and structure. The ValRS-EF-1H complex should provide a structural support for the functional interaction of ValRS with EF-1α. A synoptic model is outlined in Fig. 6. ValRS is tightly associated to the β, γ, and δ subunits of EF-1H. EF-1α is loosely bound to this complex: (i) it is easily dissociated from the other components following chromatography on a Mono Q column (21) or following incubation with aminocyl-tRNA and GTP (17); (ii) catalytic amount of the ValRS-EF-1H complex can efficiently exchange GTP for GDP from a 100-fold molar excess of EF-1α-[3H]GDP (Fig. 3). Therefore, EF-1α-GTP can be recycled into EF-1α-GDP by the exchange factors bound to ValRS even in the absence of synthetase activity, at least in vitro (Fig. 6, top right). In parallel, the ValRS component of the complex is able to catalyze tRNA\textsuperscript{Val} aminoacylation even in the absence of GTP or of an excess of free EF-1α, that is when the elongation factors are not functioning (Fig. 6, top left). However, the concomitant functioning of ValRS and of the EF-1 subunits is accompanied by a 2-fold increase in the elongation rate. Since the $k_{\text{on}}$ parameter is primarily affected, the EF-1α-GTP facilitated ValRS activity is most likely the result of a protein-protein interaction that induces a conformational change in ValRS, and promotes the release of Val-tRNA\textsuperscript{Val} from the enzyme, suggesting that product release is the rate-limiting step. Coupling of these two parallel reactions would favor the direct transfer of the aminoacylated tRNA to the elongation factor, with formation of the ternary complex EF-1α-GTP-Val-tRNA\textsuperscript{Val} (Fig. 6, bottom). In vivo, this coupling could be responsible for the channeling of tRNA reported by Deutscher and co-workers (8, 10). Using a permeabilized cell system, they observed that exogenously added tRNA or aminocyl-tRNA are not effective precursors for protein synthesis, suggesting that there is a channeled tRNA cycle in mammalian cells. In the case of the ValRS system, our results suggest that the release of the aminoacylated tRNA from the synthetase could be controlled by the availability of free EF-1α, therefore providing a rational explanation for channeling. In that connection, it should be stressed that a great deal of data reports that members of the EF-1H and of the ValRS-EF-1H complexes are the targets of phosphorylation events (21, 29–32). Hyperphosphorylation of elongation factor 1α is also observed in virus infected cells (33). The functional significance of these modifications is poorly understood but could alter translational efficiency. Whether these posttranslational modifications have a role on the EF-1α-induced stimulation of ValRS, and therefore on tRNA channeling in vivo remains to be shown, but could be a means to regulate the efficiency of tRNA delivery for protein synthesis.

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FIG. 6. Channeling of tRNA\textsuperscript{Val} in the valyl-tRNA synthetase-elongation factor 1H complex. The ValRS-EF-1H complex is a dimer of an elementary core made of equimolar amounts of each subunit. For clarity, only one monomeric core containing one copy of ValRS and of the α, β, γ, and δ subunits of EF-1 is shown. Two parallel reactions take place on this complex: specific recognition of tRNA\textsuperscript{Val} by the synthetase followed by its aminoacylation by valine and association of ValRS activity and the adequate association of ValRS and EF-1. The EF-1α-GTP-dependent stimulation of tRNA\textsuperscript{Val} aminoacylation suggests that these two reactions are concerted mechanisms. Formation of the ternary complex EF-1α-GTP-Val-tRNA\textsuperscript{Val} on the ValRS-EF-1H complex would be responsible for the vectorial transfer of tRNA from the synthetase to the ribosome.