Overproduced rho factor from p39AS has lysine replacing glutamic acid at residue 155 in the linker region between its RNA and ATP binding domains

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Rho protein is an important factor affecting transcription termination in *E.coli* (1). Studies of rho have been facilitated by cloning the structural portion of the rho gene into a vector, p39AS, permitting induced expression at very high levels (2). At that time, we noted 'a very slight mobility difference between rho protein produced in the strain containing the original rho clone and that purified from W3110', located within the amino terminal f1 fragment, and suggested that 'this simply reflects an allelic difference between strains that has no functional significance' (2). Subsequently, tryptic peptides of rho protein isolated from a strain carrying the overproducing vector p39AS were separated on a reverse phase C8 column, and then sequenced. Among the fragments analyzed was one whose alignment indicated a tryptic cleavage after amino acid 155, thought to be glutamic acid. Sequence analysis of the rho gene carried by p39AS confirmed that the GAA codon at position 155 had become AAA, encoding lysine, which also explained the shifted isoelectric point of p39AS protein relative to rho from a strain not carrying the vector (C.Burns and J.P.Richardson, unpublished). Many of the major activities of rho and a large number of rho mutants have been analyzed using this lysine-containing variant, now termed EK155, obtained from the overproduction vector p39AS. We have corrected the error in the p39AS vector, reintroducing a lysine at position 155 by site-directed mutagenesis and confirming by sequence analysis the integrity of the entire coding region, and have compared the properties of EK155 to the *bona fide* rho protein.

Most of the properties of rho EK155, including its transcription termination activity *in vitro*, are indistinguishable from those of the wild-type protein. The one main difference we find is a two-fold activation of RNA-dependent ATPase activity by the natural RNA substrate, *trp t'*, a region of rho-dependent termination about 280 bp downstream from the last structural gene of the *trp* operon in *E.coli*. Although the rates of helicase activity under optimal conditions (see 1, 3) are too fast to see any differences between the two proteins (> 70% duplex disruption in less than 30 sec.), when the reaction rate is inhibited by shifting the magnesium:ATP ratio to 5:1 (1 mM:0.2 mM), we observe a two-fold faster helicase activity for the wild-type rho protein. However, despite the increased level of *trp t'*-activated ATPase, the responses of wild-type and EK155 proteins using poly(C) RNA as an activator of ATP hydrolysis are indistinguishable. The two proteins also have the same measured affinity for *trp t'* RNA (9.5 ± 0.2 pM, under our conditions) and a conserved

percentage of binding energy contributed by ionic versus non-ionic interactions, as judged by RNA binding curves carried out at different salt concentrations (see 3).

As amino acid 155 lies in a putative hinge region between rho's RNA and ATP binding domains, the mutated residue could hypothetically participate in helping to couple the two activities of RNA-binding and ATP-hydrolysis. It is unlikely to act directly in contacting RNA since the number of ion pairs involved in RNA binding is the same for each protein, despite a net charge difference of +2 for EK155. One possible explanation for the faster rates seen with wild-type rho is that the original glutamate facilitates conformational transitions that occur as rho binds to RNA and/or ATP. Interestingly, our preliminary comparison of partial digestion patterns indicates that the wild-type protein is somewhat more stable to challenge by trypsin, especially when bound to either substrate. It is also notable that a mutation of the adjacent Asp-156 to asparagine decreases the efficiency of rho-mediated termination without affecting RNA binding (4).

Previous results have shown that EK155 is a strong terminator of transcription, both *in vitro* and *in vivo*. Our results indicate that the two-fold higher ATPase activation of wild-type rho by *trp t'* does not otherwise affect overall function under normal conditions. We therefore believe that the fundamental conclusions in the literature about rho action, structure, and function using the vector p39AS are sound, even though based inadvertently on studies with the nearly silent mutation EK155. We believe that future research, using the *bona fide* wild-type rho, will build on this foundation and continue to advance our understanding of factor-mediated termination. The new vector, designated p39ASE, is freely available upon request to the corresponding author.

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