Direct measurement of absolute suppressor efficiency

Vishvanath NENE and Robert E. GLASS

Department of Biochemistry, Queen's Medical Centre,
Clifton Boulevard, Nottingham NG7 2UH, U.K.

(Received 4 June 1981)

We have devised a system for measuring the degree of translational readthrough past a nonsense mutation which is based upon the quantitation of the two translation products, the suppressed polypeptide and the nonsense fragment. The absolute efficiency of four different amber suppressors (Su1, Su2, Su3, and Su7) has been determined at two unique amber sites in the structural gene for the \( \beta \) subunit of Escherichia coli RNA polymerase.

Nonsense suppressors are mutant tRNA species capable of recognizing translational stop codons and, thus, of preventing premature termination of polypeptide chain elongation (reviewed in reference 1). For convenience, suppressor efficiency is often determined from the biological activity of the suppressed polypeptide alone. However, since activity is dependent upon the suitability of the inserted amino acid, the ability of a suppressor tRNA to function at a particular site is dictated by the nature of the amino acid inserted as well as the efficiency of its insertion. To measure the absolute suppressor efficiency – by which we mean the frequency of translational readthrough – it is necessary to quantitate both translation products of the mutant gene, namely, the prematurely terminated fragment and the suppressed polypeptide.

We have characterized two mutant Escherichia coli strains harbouring unique amber lesions in the C-terminal region of \( rpoB \), the structural gene for the \( \beta \) subunit of RNA polymerase. They synthesize N-terminal fragments of molecular weight 138 500 and 123 000 (the size of the wild-type \( \beta \) subunit is calculated to be 150 618 daltons from the DNA sequence (2)). Since the majority of monomeric protein species in E. coli are smaller than 100 000 daltons (3), it is practical to quantitate both the \( \beta \) fragment and the suppressed polypeptide by electrophoretic separation of total cellular proteins in SDS-polyacrylamide slab gels. We thus have a system ideally suited for measuring absolute suppressor efficiencies and the influence that other factors such as a reading context may have on translational readthrough.

Methods

The genotypes of the \( rpoB \) amber strains are given in the legend to Table 1. Lysates of Su1, Su2, Su3, and Su7 suppressed derivatives were prepared at 30°C as described previously except that a 3.0-min
Table 1. Synthesis of β subunits and β fragments in the presence of four different amber suppressors

<table>
<thead>
<tr>
<th>Strain (relevant genotype)</th>
<th>β subunit</th>
<th>β fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B12</td>
<td>B1603</td>
</tr>
<tr>
<td>AJ1 (rpoB⁺)</td>
<td>0.30 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>AJ5021 (rpoB12(Am) supD (Su1))</td>
<td>0.28 ± 0.02</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>AJ5022 (rpoB12(Am) supE (Su2))</td>
<td>0.17 ± 0.01</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>AJ5023 (rpoB12(Am) supF (Su3))</td>
<td>0.40 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>AJ5027 (rpoB12(Am) supU (Su7))</td>
<td>0.23 ± 0.04</td>
<td>0.43 ± 0.11</td>
</tr>
<tr>
<td>AJ6031 (rpoB1603(Am) supD (Su1))</td>
<td>0.29 ± 0.02</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>AJ6032 (rpoB1603(Am) supE (Su2))</td>
<td>0.14 ± 0.02</td>
<td>1.05 ± 0.20</td>
</tr>
<tr>
<td>AJ6033 (rpoB1603(Am) supF (Su3))</td>
<td>0.28 ± 0.04</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>AJ6037 (rpoB1603(Am) supU (Su7))</td>
<td>0.32 ± 0.02</td>
<td>0.30 ± 0.04</td>
</tr>
</tbody>
</table>

Pulse-and-chase period was employed; such labelling conditions ensure minimal degradation of labile proteins (3). Radiolabelled proteins were fractionated on 28-cm-long, 5% SDS-polyacrylamide slab gels (4) at a constant current of 10 mA for 20 h. The gel tracks were excised, after visualization of protein bands by staining, and 1-mm slices counted for radioactivity as before (4). The larger amber fragment has been previously characterized (3); further details on this truncated polypeptide and the smaller species will be presented elsewhere.

Results and Discussion

In strains carrying amber lesions in rpoB, each productive translational initiation event on the β mRNA can give rise to either a complete β polypeptide or an N-terminal fragment, depending upon whether there is translational readthrough past the nonsense site. The efficiency of suppression of premature translational termination can, therefore, be determined from the proportion of complete β chains relative to the number of translational initiation events (i.e. the total number of β and β-fragment molecules). The large amber fragments synthesized by mutants carrying the rpoB12 and rpoB1603 nonsense lesions (Table 1) has allowed us to determine the absolute efficiency of Su1, Su2, Su3, and Su7 at two distinct sites (Table 2).
Table 2. The absolute efficiency of suppression of rpoB12(Am) and rpoB1603(Am)

<table>
<thead>
<tr>
<th>Suppressor</th>
<th>rpoB12(Am)</th>
<th>rpoB1603(Am)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Su1</td>
<td>58</td>
<td>37</td>
</tr>
<tr>
<td>Su2</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Su3</td>
<td>69</td>
<td>66</td>
</tr>
<tr>
<td>Su7</td>
<td>34</td>
<td>52</td>
</tr>
</tbody>
</table>

Absolute suppressor efficiency (degree of translational read-through)

\[
\text{Absolute suppressor efficiency} = \frac{\text{total number of suppression events}}{\text{total number of translation initiations}}
\]

\[
= \frac{\text{Per cent total } ^3\text{H in } \beta}{\text{percent total } ^3\text{H in } \beta + \text{percent total } ^3\text{H in } \beta \text{ fragment}} \times 100
\]

(Net note that initiation in this instance refers to events that occur at the normal $\beta$ ribosome binding site and not internal starts.)

The four amber suppressors vary in efficiency over about a six-fold range (12 to 69%; Table 2). The fact that Su3 is the most efficient and Su2 the least efficient suppressor at the two mutant sites is consistent with previous data (5). The ranking order of the suppressors, Su2 < Su7 < Su1 < Su3 and Su2 < Su1 < Su7 < Su3 for rpoB12(Am) and rpoB1603(Am), respectively, demonstrates the importance of the reading context for nonsense suppression. This contention is further supported by the difference in translational readthrough frequency for a single suppressor at the amber sites: only the strongest suppressor, Su3, appears to function largely independently of the reading context.

Our system is amenable to analysis of parameters, such as ribosomal discrimination (6), which can be imposed upon the translational event. For example, the strains employed carry a rpsL (Str-r) allele that appears to restrict suppressor function (see reference 7) at the readthrough level since addition of streptomycin to the growth medium increases translational readthrough (V. Nene & R.E. Glass, manuscript in preparation). Moreover, although it has been possible to measure suppressor function in the past by quantitating the relative activity of the suppressed polypeptide or its ability to fulfill a structural role in a hetero-oligomer (see for instance 8), such procedures cannot take into consideration transcriptional or translational controls on gene expression. Measurement of both translation products allows for regulatory effects (the implications of the results on RNA polymerase control will be discussed elsewhere).

The system described is applicable only to the study of amber suppressors. We are at present in the process of obtaining ochre derivatives of these large-fragment-producing strains.
Acknowledgements

We are most grateful to all those who sent strains. We thank Lepetit Pharmaceuticals Ltd., Maidenhead, for a gift of rifampicin. This work was supported by an MRC Project Grant.

References