Enhanced expression of recombinant proteins in insect cells using a baculovirus vector containing a bacterial leader sequence

Timothy C. Peakman, Ian G. Charles, Mark A. Sydenham, Dirk R. Gewert, Martin J. Page* and Andrew J. Makoff
Department of Cell Biology, Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS, UK

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Baculovirus/insect expression systems have been in use for several years, often resulting in very high levels of expression of recombinant proteins (1). For expression of mature proteins the preferred vectors, such as pVL9141 (2) or p36C (3), utilise the strong polyhedrin promoter and leader sequence. We recently reported the finding that the presence of a 24 bp bacterial sequence immediately upstream of the initiation codon unexpectedly supported high expression levels of tetanus toxin fragment C (4). The bacterial leader sequence is derived from the 3' end of the E. coli trpE gene where it functions as part of the ribosome binding site of the adjacent trpD gene (5). This sequence also enabled P69 pertactin from Bordetella pertussis to be expressed at high levels by baculovirus-infected cells (unpublished observations). We have investigated this bacterial sequence further, by comparing its effect on the expression of three different heterologous genes.

A 40 kDa fragment (P40) from B. pertussis, which is found in purified extracts of P69 pertactin (6) and which contains the main antigenic determinants of the P69 molecule (7) was expressed in the insect/baculovirus system. Two constructs were made: either with (vAc36CP40-pl) or without (vAc36CP40) the 24 bp bacterial leader sequence (Figure 1), in both cases by ligating the P40 part of the P69 pertactin gene and an appropriate oligonucleotide into the BamHI site of p36C (3). From these transfer vectors were generated recombinant baculovirus as previously described (8).

Both recombinant baculoviruses were used to infect Spodoptera frugiperda cells. In common with other higher eukaryotic expression systems, variation in expression levels between different batches of insect cells, infected by the same recombinant baculovirus, can be quite large. Consequently, both recombinant baculoviruses were used to infect the same batch of cells at the same time. Both sets of infected cells were harvested after 72 hours and expression levels were analysed by densitometer scanning of stained SDS–polyacrylamide gels. The presence of the bacterial leader sequence increased expression by a factor of 2 (Table 1). This comparison was repeated by infections of a different batch of insect cells and again the presence of the bacterial leader sequence resulted in the higher expression by a similar factor (data not shown).

The metalloproteinase enzyme stromelysin (9) was also expressed using two analogous constructs (Figure 1). Expression of stromelysin was much lower than P40 and was quantitated by assaying its protease activity. Again the bacterial leader stimulated expression two-fold (Table 1). Again a repeat pair of infections gave a similar stimulation (data not shown).

<table>
<thead>
<tr>
<th>Recombinant protein</th>
<th>Vector</th>
<th>Bacterial leader sequence</th>
<th>Expression level (%) total cell protein</th>
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<tbody>
<tr>
<td>P40</td>
<td>vAc36CP40</td>
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<td>4.7</td>
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<td>vAc36CST1</td>
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<td></td>
<td>vAc36CSTM2</td>
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<td>Stromelysin</td>
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<tr>
<td></td>
<td>vAc36CSTM2</td>
<td>yes</td>
<td>27.2</td>
</tr>
</tbody>
</table>

* To whom correspondence should be addressed

+Present address: Medeva Group Research, Vaccine Research Unit, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7, UK
The final recombinant protein to be investigated was integrase from HIV type I (10). In this experiment, the bacterial leader sequence was directly compared with a consensus sequence optimised for vertebrates (11), since it was conceivable that such a sequence might also function well in an invertebrate system (Figure 1). Expression levels were very high, with the bacterial leader construct achieving nearly 30% total cell protein, approximately five times the level achieved by the consensus sequence (Table 1). For this gene at least, the consensus sequence is clearly not optimal for insect/baculovirus expression, perhaps because of its very high GC content.

In order to find out whether the bacterial sequence was acting at the level of transcription or translation, RNA was isolated from each infected cell culture. Comparative levels of the recombinant mRNA was assessed by slot blots using a probe which hybridized to a sequence immediately downstream of the foreign gene and common to all six constructs. The amount of mRNA produced by these constructs was almost identical, and failed to reflect the variation in expression within each pair (data not shown). This strongly suggests that the bacterial sequence is not acting on transcription or on mRNA turnover, and therefore primarily affects the efficiency of translation.

Recently, Covener and Ray (12) analysed the nucleotide frequencies around the initiation codons of a large number of eukaryotic genes. For invertebrates, this analysis generates the consensus sequence A/C A A A/C [A U G] A/G for the four positions upstream and one position downstream of the AUG. In common with all classes of eukaryotic sequence, there is a very strong bias for a purine (particularly A) at position −3. Interestingly, for each of the three genes described in this work, the construct containing the 24 bp bacterial leader sequence has a worse fit to this invertebrate consensus than the other construct (Figure 1), and the −3 position is a C. Our results therefore suggest that this consensus sequence does not have any relevance for baculovirus-infected insect cells, perhaps because viral infection has some effect on the host translational machinery.

The 24 bp bacterial leader sequence appears to offer significant improvements in expression of foreign genes in baculovirus-infected insect cells.

REFERENCES