Comparison of *Schizosaccharomyces pombe* expression systems

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Analysis of a variety of problems in yeast relies on ectopic expression of the protein of interest under control of a heterologous promoter. In the fission yeast *S. pombe*, there are relatively few expression plasmids and most reports of their activity have not been comparative. It is thus difficult to determine the comparability of experiments carried out using different expression vectors. When new promoters are characterised, it is likewise difficult to determine their activity relative to those previously identified. In order to better assess relative promoter strengths, I have compared the activity of several inducible and constitutive expression systems in fission yeast by using a beta-galactosidase reporter gene. This allows a more informed choice of appropriate expression systems for analysis of gene function in *S. pombe*.

Four regulatable and two constitutive promoters were compared. Three regulated promoters were derived from the powerful nmt1 promoter, first described by Maundrell (1) and subsequently attenuated with mutations in the TATA box by Basi et al. (2). The other regulated system was constructed from the tetracycline-inducible system described by Faryar and Gatz (3). The two constitutive promoters were the previously described vectors pART1 (containing the adh promoter; (4)) and pSM1 (containing the SV40 promoter; (5)).

The expression vector REP3, containing the thiamine-inducible nmt1 promoter (1), and its derivatives REP41 and REP81 (2), which have lower levels of activity due to mutation, all contain an ATG within their polylinker. This was destroyed by insertion of a Xho linker; these derivatives are called REP3X (full strength nmt1), REP41X (slightly weaker; nmt1*) and REP81X (much weaker; nmt1**). The tetracycline expression system was originally described in (3) and modified for more general use. The expression vector, pSLF101, has the constitutive CaMV promoter adjacent to the tet operator as described in (3); in addition, it contains a polylinker, and the LEU2 and ars1 markers for selection in fission yeast. A ura4+ marked version, pSLF102, was also constructed but not tested. The tet repressor, which blocks transcription from the otherwise constitutive CaMV promoter, was provided by integration of plasmid pSLF104 into a strain of genotype h- ade6-704 leu1-32 ura4-D18. The plasmid contains the marker sup3-5, which suppresses ade6-704, and the tet repressor gene under control of the *S. pombe adh* promoter, as described by Faryar and Gatz (3). The resulting fission yeast strain, FY191, thus produces a constitutive tetracycline repressor protein.

A 3 kb BamHI cassette containing the beta-galactosidase gene (6) was cloned into the BamHI site in the polylinkers of all the vectors, and transformed into a fission yeast strain of genotype h- leu1-32 ura4-D18. Media and methods were as described in (7). The tet-inducible pSLF101-lacZ was also transformed into the strain with the integrated tetracycline repressor. Beta-galactosidase assays were carried out as described in (8). All assays were carried out after growth for 24 hrs at 30°C on strains grown to mid-exponential phase in EMM + uracil (7) with additional supplements as follows. The REP vectors (REP3X-lacZ, REP41X-lacZ, and REP81X-lacZ) were grown in 5 μg/ml thiamine for repression and induced by growth in normal EMM. The CaMV-tet vector (pSLF101-lacZ) in the strain FY191 was repressed in normal EMM, and induced by addition of

### Table I. Beta-galactosidase activity of different *S. pombe* expression systems

<table>
<thead>
<tr>
<th>Vector</th>
<th>promoter</th>
<th>Repressed Conditions b</th>
<th>Induced Conditions b</th>
<th>Approx. Induction Ratio</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pART1-lacZ</td>
<td>adh</td>
<td>NA d</td>
<td>4049 ± 1195</td>
<td>constit c</td>
<td>(4)</td>
</tr>
<tr>
<td>pSM-lacZ</td>
<td>SV40</td>
<td>NA</td>
<td>8.1 ± 1.3</td>
<td>constit</td>
<td>(5)</td>
</tr>
<tr>
<td>pSLF101-lacZ</td>
<td>+ tet repressor</td>
<td>CaMV-tet</td>
<td>0.9 ± 0.2</td>
<td>10.6 ± 2.5</td>
<td>100X</td>
</tr>
<tr>
<td></td>
<td>- tet repressor</td>
<td>CaMV-tet</td>
<td>9.6 ± 3</td>
<td>9.7 ± 2.5</td>
<td>constit</td>
</tr>
<tr>
<td>REP3X-lacZ</td>
<td>nmt1 full strength</td>
<td>CaMV-tet</td>
<td>24.7 ± 9</td>
<td>7395 ± 404</td>
<td>300X</td>
</tr>
<tr>
<td>REP41X-lacZ</td>
<td>nmt1 weaker</td>
<td>CaMV-tet</td>
<td>5.1 ± 1</td>
<td>121 ± 30</td>
<td>25X</td>
</tr>
<tr>
<td>REP81X-lacZ</td>
<td>nmt1 weakest</td>
<td>CaMV-tet</td>
<td>1.2 ± 0.3</td>
<td>7.2 ± 2</td>
<td>7X</td>
</tr>
</tbody>
</table>

**a**Units = (1000 × OD420 × (min × ml × OD₅₅₀)⁻¹. **b**Repressed conditions: for PSLF101: EMM; for REP3X, 41X, and 81X, EMM + 5 μg/ml thiamine. **c**Induced conditions: for PSLF101: EMM + 10 μg/ml tetracycline; for REP3X, 41X, and 81X: EMM. **d**NA: not applicable (constitutive promoters). **e**Constit: constitutive promoter.
tetracycline to 10 μg/ml; there was no difference between 10 and
100 μg/ml tetracycline (data not shown). Activity of the CaMV-
tet system in the absence of the integrated tet-repressor, where
it should be constitutive, was also determined. pART1-lacZ and
pSM-lacZ were grown in normal minimal medium. Units of beta-
galactosidase activity were determined as described (8) on
duplicate transformants. The averages of these assays are shown
in Table 1.

As seen in the table, the full-strength nmt1 promoter in REP3X-
lacZ provides a dramatic induction, and has substantial levels
of expression even under repressed conditions. The attenuated
versions REP41X-lacZ and REP81X-lacZ as reported in (2) have
reduced activity in both repressed and induced conditions. The
absolute induction ratios and relative activity between the three
REP vectors and adh promoter reported in Basi et al. (2) differ
somewhat from those reported here. This is almost certainly due
to the difference in reporter systems; Basi et al. used the CAT
gene as a reporter and in this study, lacZ was the reporter gene.

Beta-galactosidase is a stable enzyme, and obviously the
translation context and the stability of the protein being expressed
will influence the actual levels of protein in the cell. This is true
of any reporter system, and offers a necessary caveat in the
interpretation of these data. The induced levels of the tet
responsive plasmid pSLF101-lacZ are comparable to those of the
weakest nmt1 vector REP81X-lacZ. The S. pombe adh promoter
has a constitutive expression level similar to the induced levels
of the strongest nmt1 promoter, and the SV40 promoter has
constitutive levels similar to those of the induced tet system, or
the weakest nmt1. In the absence of tetracycline repressor,
pSLF101-lacZ using the CaMV promoter is expressed
constitutively at levels similar to the SV40 promoter.

These results should assist in the choice of promoter for
functional studies using ectopic expression in the fission yeast.
As determined using a beta-galactosidase reporter system, the
different plasmids can produce constitutive or regulated
expression at a range of levels from low to very high.
Additionally, these data should allow a direct comparison of
activity when other fission yeast promoters are characterised in
the future.

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