Journal of Biotechnology 161 (2012) 265–268

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

Protein expression can be monitored in yeast by peptide-mediated induction of TetR-controlled gene expression

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ARTICLE INFO

Article history:
Received 16 December 2011
Received in revised form 17 April 2012
Accepted 5 May 2012
Available online 20 June 2012

Dedication: This manuscript is dedicated to the memory of our highly valued mentor Wolfgang Hillen, a continuous source of inspiration. Wolfgang Hillen died unexpectedly in October 2010 and will be dearly missed.

Keywords:
TetR
TetR-inducing peptides
TIP
Gene expression analysis
Yeast

ABSTRACT

The rapidly increasing number of completed genome sequences urgently calls for convenient and efficient methods for analysis of gene function and expression. TetR-inducing peptides (TIP) can induce reporter gene expression controlled by Tet repressor (TetR) when fused to a protein of choice which makes them a highly valuable tool for monitoring expression *in vivo*. However, TIP functionality has only been demonstrated in bacteria so far. Here, we report that TIP is also functional in yeast. An mCherry-TIP fusion that locates to the nucleus induces TetR-controlled *gfp+* expression in a dose-dependent manner. This opens up potential applications in proteome research in which the expression of proteins can be analyzed *in vivo* by fusing TIP to proteins of choice in conjunction with a Tet-controlled reporter system.

The availability of complete genome sequences for many different organisms has shifted the focus of research activity towards functional analysis of the genome-encoded biomolecules. The analysis of the proteome is of particular interest due to its highly dynamic nature which allows cells to rapidly react and adjust to a changing environment. To gain insight into these processes, experimental approaches generally combine 2D-gel electrophoresis with mass spectrometric analysis. Both can be time-consuming and cumbersome. The limitations of such approaches are well-known and include, *inter alia*, difficulties in detecting proteins of low abundance, proteins with pI values beyond the typical range used during isoelectric focusing or hydrophobic proteins (reviewed in Garbis et al., 2005). Unfortunately, the expression analysis of low abundance proteins, which often include transcription factors, is of particular interest since they frequently represent potential drug targets or disease biomarkers. Hence, alternative and more convenient methods allowing a direct *in vivo* read-out of their expression would be advantageous.

We recently isolated and characterized a group of peptides that interact with the bacterial tetracycline repressor (TetR) to induce TetR-controlled gene expression *in vivo* (Klotzsche et al., 2005, 2007; Daam et al., 2008; Goeke et al., 2012). These 12- to 16-amino acid long peptides functionally replace tetracycline (tc), the natural inducer of TetR, and were shown to exert their effect by binding to the tc-binding pocket of TetR (Luckner et al., 2007; Sevvana et al., 2012). When such TetR-inducing peptides (TIP) are fused to a protein of interest, it is converted into a protein-based TetR inducer, consequently allowing its expression to be monitored *in vivo* with a TetR-controlled reporter system.

This TIP-tag has already been introduced as an efficient tool for analyzing protein expression in *Escherichia coli* and *Staphyllococcus aureus*. Transposase-mediated random insertion of the tag created in-frame translational fusions of TIP with endogenous proteins from *E. coli* (Schlicht et al., 2006). The expression patterns found were all in agreement with published data obtained by other methods. In *S. aureus*, time- and concentration-dependent expression of an mCherry-TIP fusion protein correlated with TetR-controlled GFP activity (Gauger et al., 2012). However, up to now, functionality of TIP has only been demonstrated in bacteria.

Abbreviations: TIP, TetR-inducing peptide; TetR, Tet repressor; tTS, tetracycline-dependent transsilencer; dox, doxycycline; tc, tetracycline; NLS, nuclear localization signal; pI, isoelectric point.

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http://dx.doi.org/10.1016/j.jbiotec.2012.05.012

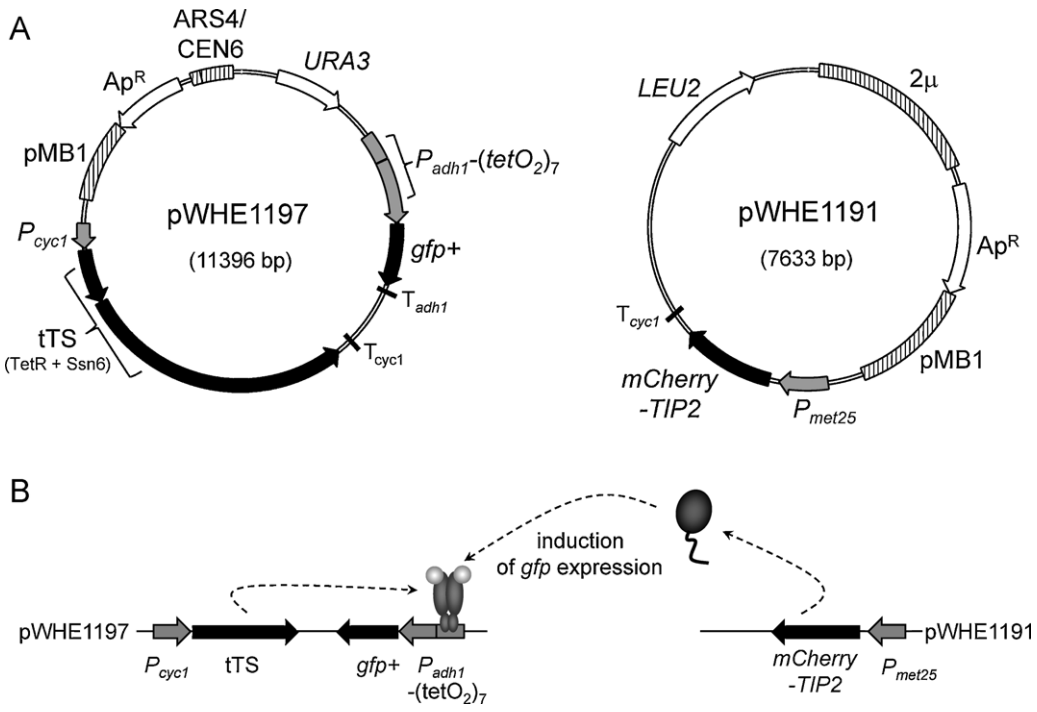

Fig. 1. (A) Architecture of yeast plasmids used to analyze TIP-mediated induction of TetR. pWHE1197 encodes for tTS consisting of TetR fused to the repression domain Ssn6 under transcriptional control of the promoter P_{cyc1}. The reporter gene *gfp+* is under control of the Tet-responsive promoter P_{adh1}-$(tetO_2)_7$. Origin of replication and selection marker for *E. coli* (pMB1, Ap^R) and yeast (ARSH4/CEN6, URA3) are indicated. pWHE1191 encodes for a C-terminal mCherry-TIP2 translational fusion under control of the regulable promoter P_{met25}. Origin of replication and selection marker for *E. coli* (pMB1, Ap^R) and yeast (2 µ, LEU2) are indicated. (B) Principle of TIP-mediated analysis of gene expression. In the presence of methionine, mCherry-TIP2 expression is repressed and tTS binds to P_{adh1}-$(tetO_2)_7$ thereby preventing *gfp+* transcription. In the absence of methionine, mCherry-TIP2 is expressed and induces tTS leading to expression of *gfp+*.

Therefore, we sought to establish TIP functionality in a eukaryote and chose *Saccharomyces cerevisiae*, one of the most intensively studied and well-characterized model organisms. For this purpose a reporter system was constructed on two compatible plasmids (Fig. 1A and Supplementary data). The first plasmid encodes TIP2, a recently isolated highly active TIP variant (Goeke et al., 2012), fused to the C-terminus of the fluorescent protein mCherry. The use of a fluorescent scaffold protein allows to quantify the TIP2 expression level and to detect its subcellular localisation. The mCherry-TIP fusion is under transcriptional control of the *S. cerevisiae* promoter P_{met25}, which can be repressed with methionine (Kerjan et al., 1986; Sangsoda et al., 1985). Furthermore, an SV40 nuclear localization signal (NLS) was N-terminally fused to mCherry to ensure nuclear accumulation of the TIP fusion protein (Kalderon et al., 1984a,b). The second plasmid encodes GFP+ which serves as a reporter gene to score TIP-mediated induction of TetR (Scholz et al., 2000). *gfp+* is under transcriptional control of the promoter P_{adh1} (Russell and Hall, 1983) which was made Tet responsive by upstream insertion of seven $tetO_2$ boxes (Gossen and Bujard, 1992). Repression of GFP+ expression is mediated by a tetracycline-dependent trans-silencer (tTS) consisting of TetR fused to the yeast repression domain Ssn6 (Belli et al., 1998a,b). The *S. cerevisiae* P_{cyc1} promoter ensures low-level constitutive expression of this tTS variant (Mumberg et al., 1995). The use of two fluorescent proteins with distinct excitation and emission parameters facilitates the simultaneous quantification of inducer and reporter (see Supplementary data for details).

To characterize the regulatory properties of this reporter strain, the tTS variant was induced with increasing amounts of the tc derivative doxycycline (dox) in the absence of mCherry expression. At concentrations higher than 0.025 µM dox, the strain displayed a constant, dose-independent GFP+ fluorescence (Supplementary Figure). In the following measurements, 0.5 µM dox was used as a control to ensure full induction. The maximal fluorescence in the fully induced state is about 2.2-fold higher compared to the uninduced state (Fig. 2). This narrow window of regulation is attributed to the low-level expression of tTS mediated by the weak P_{cyc1} promoter since our primary goal was to create a highly sensitive reporter system to prove whether TIP-mediated induction of TetR

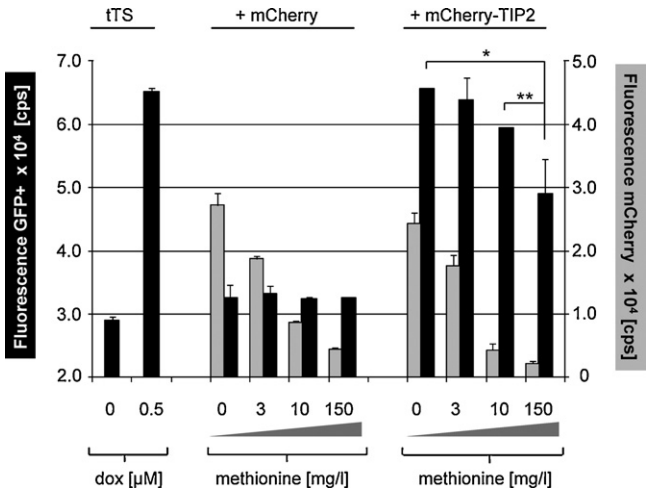

Fig. 2. Fluorescence measurement to analyze TIP-mediated induction of tTS in yeast. GFP+ fluorescence is indicated with black bars (axis on the left-hand side), mCherry fluorescence with grey bars (axis on the right-hand side). In the absence of dox, GFP+ expression is reduced to its basal level. In the presence of dox, the tetracycline-dependent trans-silencer (tTS) is induced and full expression of *gfp+* occurs. mCherry expression is regulated by the methionine-dependent promoter P_{met25} and maximal expression occurs in the absence of methionine. Expression of mCherry without TIP2 has no effect on tTS-regulated GFP+ expression. When TIP2 is fused to mCherry, GFP+ expression is fully induced in the absence of methionine. $^*P < 0.02$, $^{**}P < 0.05$ (indicated data groups were compared by an unpaired *t* test).