SELECTED OPPORTUNITIES IN GENE THERAPY

Improved vector for driving the targeted integration of a transgene into an eukaryotic genome

BIO 15039

Product factsheet

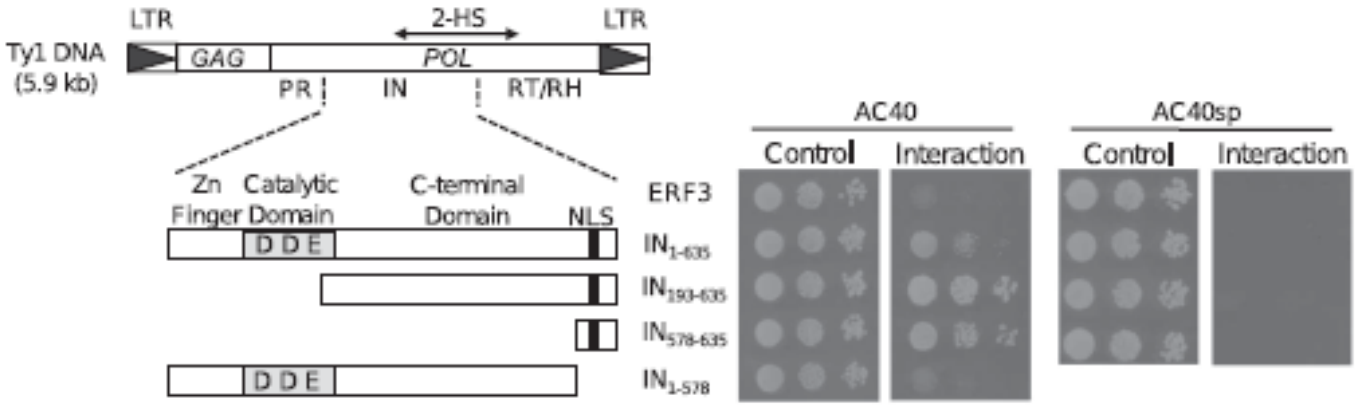
In vitro

- **Product:**
  - Polypeptide for engineering chimeric integrase proteins
- **Application:**
  - Retroviral vector based gene therapy
  - Achievement of stable transgene expression while minimizing insertional mutagenesis
- **POC:**
  - Identification of a functional interaction between Ty1 integrase (Ty1 IN) and the AC40 subunit of RNA Polymerase III by co-immunoprecipitation assay and two hybrid assay
  - Loss of interaction between AC40 and Ty1 IN not only dramatically alters integration upstream of all tDNA genes, but also induces an unexpected redistribution of Ty1 insertions to subelomeric regions (Deep sequencing of de novo insertion events)
  - Characterization and identification of the minimal Ty1 IN Targeting Domain sequence by two-hybrid assay
- **Patent and publication:**
  - WO2016128549 published on August 18, 2016
  - Bridier-Nahmias A *et al.*; Science; may 2015

Proof of concept

An RNA polymerase III subunit determines sites of retrotransposon integration

Ty1 IN interacts with AC40 but not AC40sp



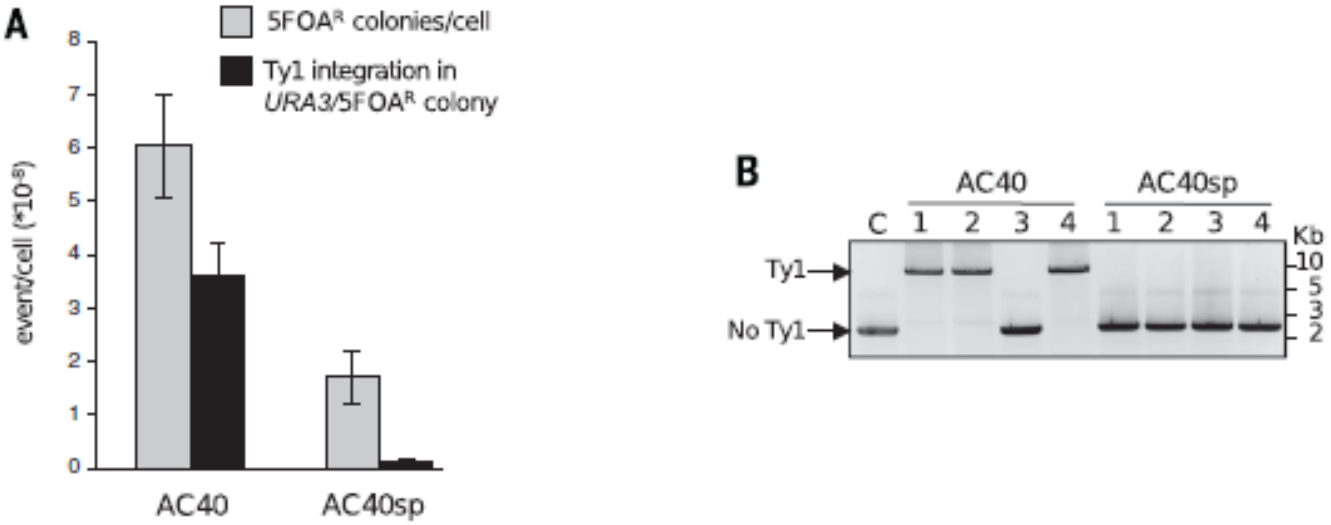
- AC40 is a cofactor of Ty1 IN
- C-terminus of IN is necessary and sufficient for the interaction
- AC40sp does not interact with Ty1 IN
  - (loss of interaction mutant)

Two-hybrid interaction between Ty1-IN and AC40 or S. pombe ortholog AC40sp.
(**Left**) The two-headed arrow indicates Ty1 sequences recovered in the two-hybrid screen (2-HS) with AC40 as bait. Different Ty1-IN regions were fused to the Gal4 activation domain; AC40 or AC40sp were fused to the Gal4 Binding Domain. (**Right**) Cells were plated onto nonselective (Control) or selective (Interaction) media to detect interactions.

Proof of concept

An RNA polymerase III subunit determines sites of retrotransposon integration

The AC40-IN interaction is important for Ty1 targeting upstream of tRNA genes



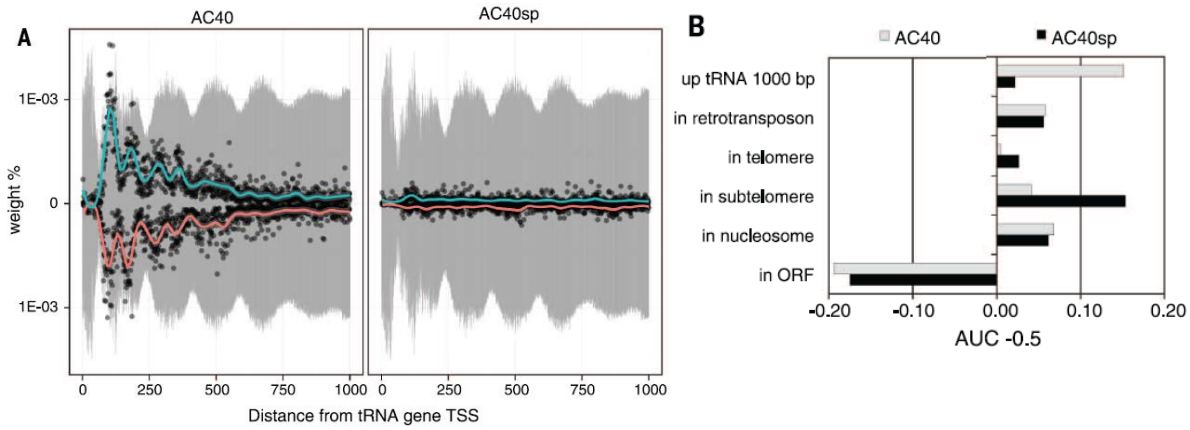
(**A**) Ty1 insertions upstream of tG(GCC)B locus, a hotspot of Ty1 integration, inactivate URA3, resulting in Ura$^-$ cells that grow on 5FOA plates. Endogenous Ty1 retrotransposition was induced in rpc40^D strains expressing AC40 or AC40sp at similar levels. (**Gray bars**) Average frequency of 5FOA^R colonies obtained from independent cultures of strains expressing AC40 and AC40sp, respectively. (**Black bars**) Ty1 insertions in URA3/5FOA^R colonies identified by PCR.
(**B**) Representative PCR analysis of 5FOA^R colonies obtained from the cultures in (A) to detect Ty1 insertions in URA3.

Proof of concept

An RNA polymerase III subunit determines sites of retrotransposon integration

Association of Ty1 insertions with chromosomal features in WT (AC40) and the loss-of-interaction mutant (AC40sp)

(A) Ty1 insertions upstream of the 275 tRNA genes of S. cerevisiae were aggregated into a single distribution with respect to distance to the start of transcription for the wild type (**AC40, left panel**) and mutant (**AC40sp, right panel**). Locally weighted scatterplot smoothing curves indicate the general trends of convergent (red) and divergent (blue) integrations relative to the tRNA gene.
(B) Association of genomic features with integration hotspots by single logistic regression models assay - A subset of representative features with positive and negative AUC – 0.5 (AUC, area under the curve values of the receiver operator characteristic). 0 indicates a model of no predictive power, 0.5 indicates a perfect prediction.

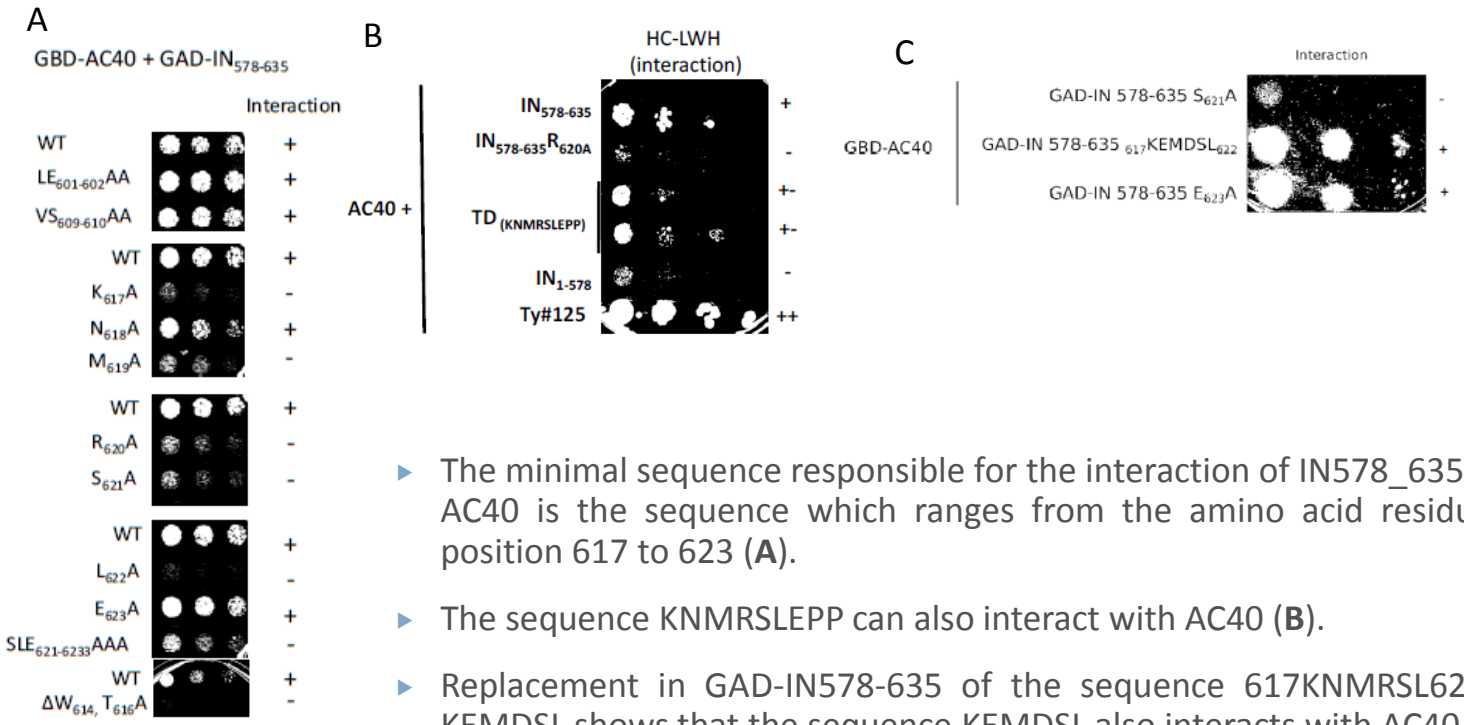


- Ty1 insertions in the WT strain were associated with upstream regions of tRNA genes or features associated with these sites, such as preexisting retrotransposons.
- The loss of interaction between AC40 and the integrase
  - dramatically alters integration upstream of all tDNA genes
  - induces an unexpected redistribution of Ty1 insertions to subelomeric regions.

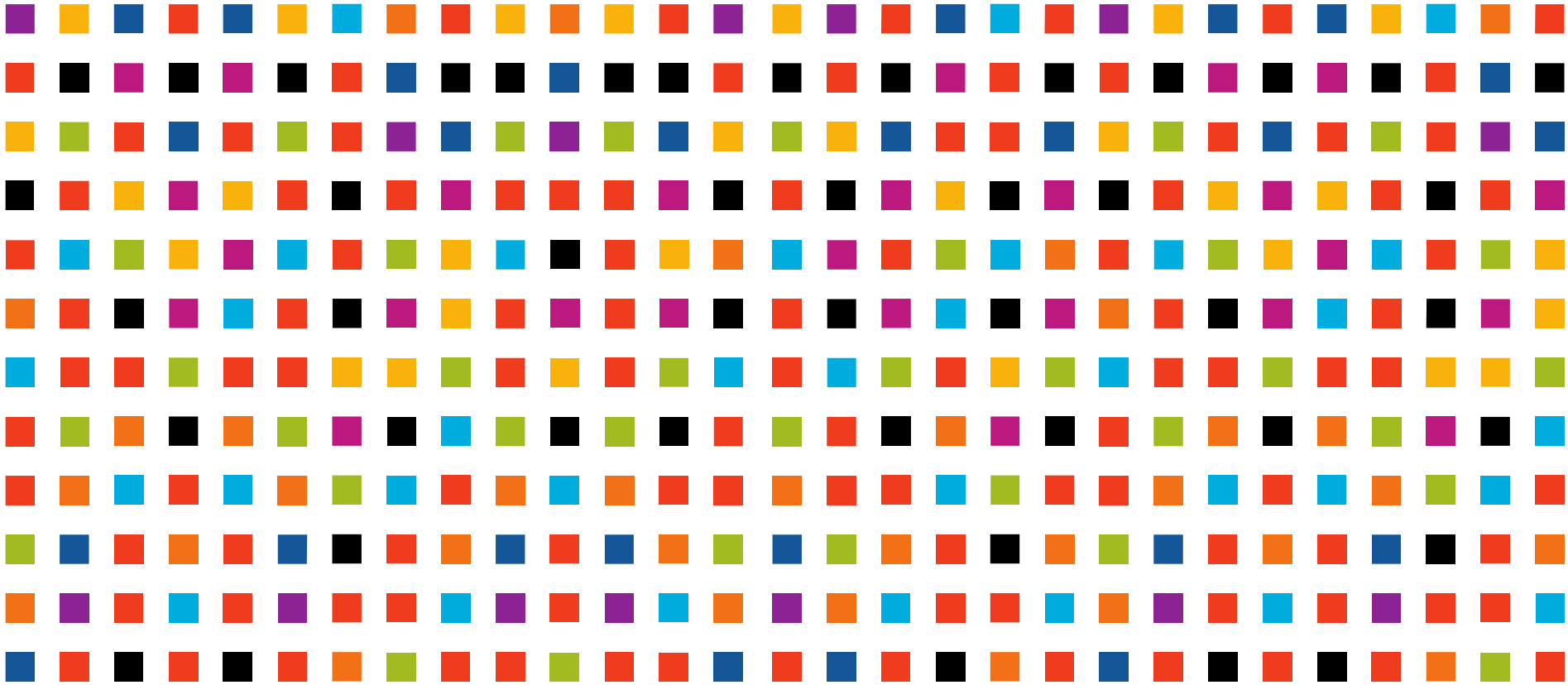
Proof of concept

Characterization of Ty1 IN Targeting Domain

Characterization of the minimal Ty1 TD by two-hybrid assay



- The minimal sequence responsible for the interaction of IN578_635 with AC40 is the sequence which ranges from the amino acid residue at position 617 to 623 (**A**).
- The sequence KNMRSLEPP can also interact with AC40 (**B**).
- Replacement in GAD-IN578-635 of the sequence 617KNMRSL622 by KEMDSL shows that the sequence KEMDSL also interacts with AC40 (**C**).



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