

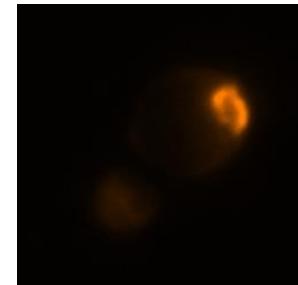
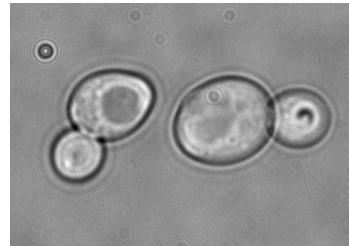


Instytut Genetyki i Biotechnologii

Wydział Biologii
Uniwersytet Warszawski



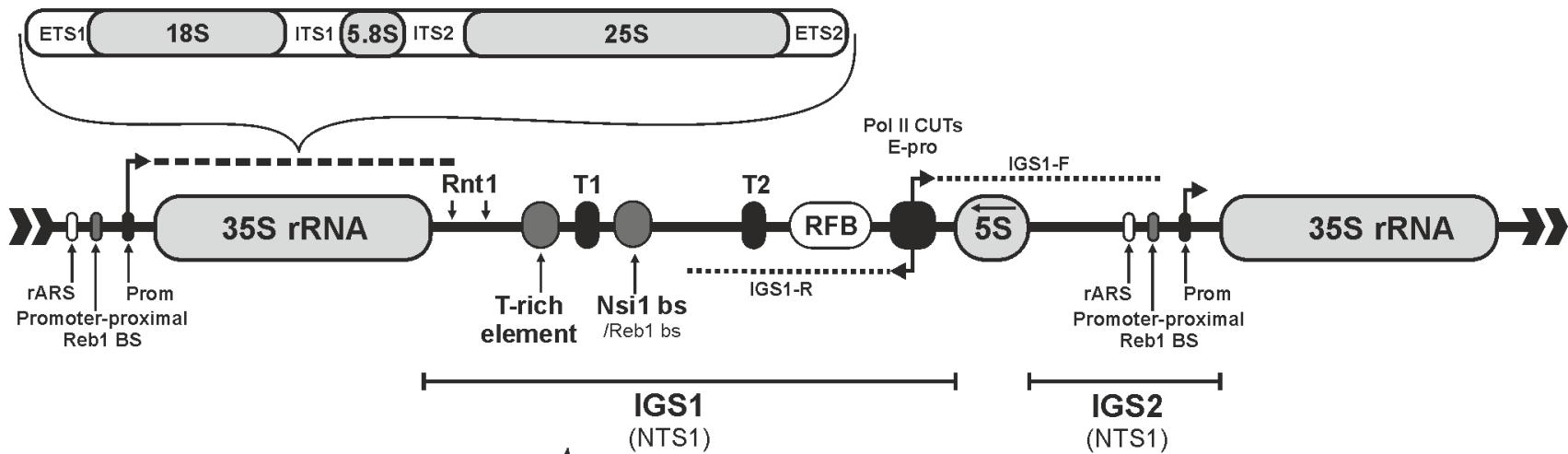
„Transkrypcja rybosomalnego RNA w komórkach drożdży: udział białek wiążących RNA Nrd1 i Nab3 w terminacji polimerazy RNA I oraz badanie struktury jąderka w mutantach z upośledzoną transkrypcją rRNA”.



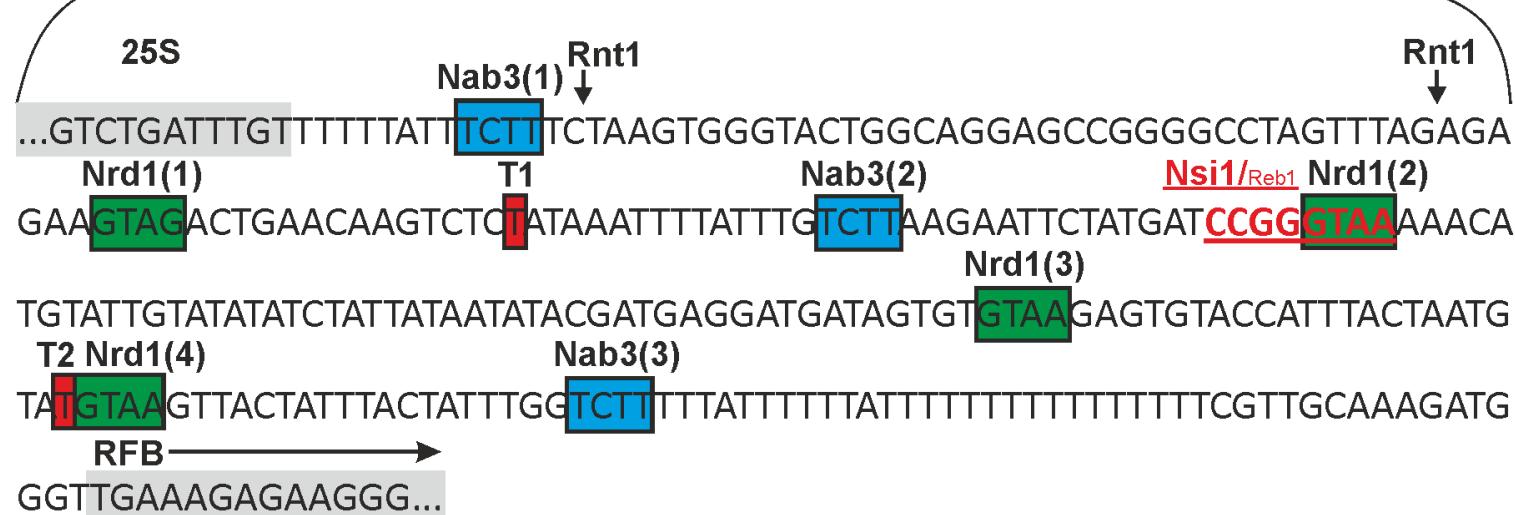
*Licencjat w IGiB UW, opiekun dr Michał Koper, grupa prof. Joanny Kufel
e-mail: mkoper@ibb.waw.pl*

Organization of rDNA loci

A

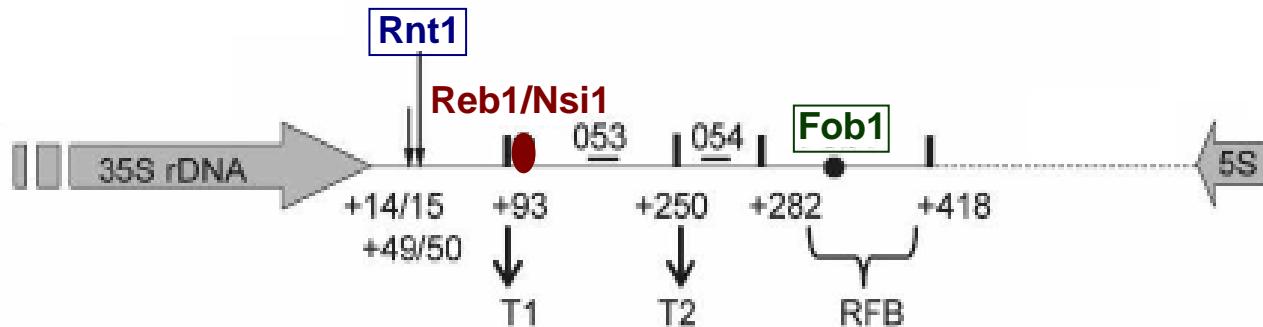


B



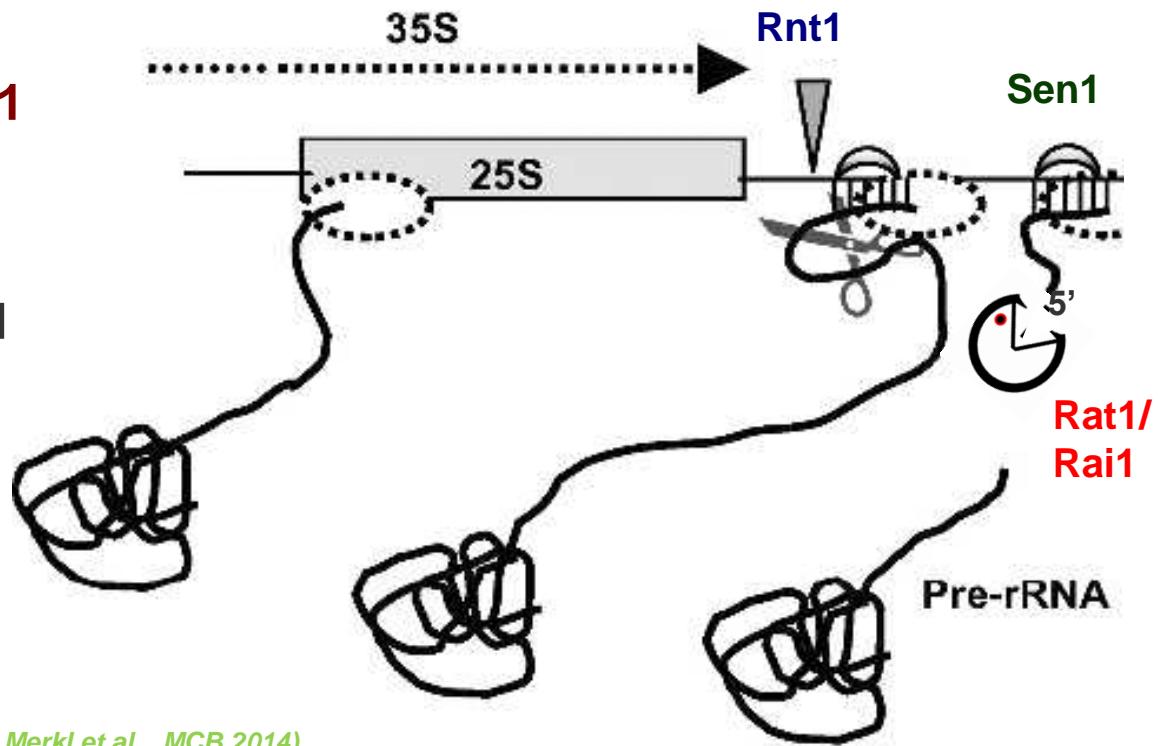
(A) Schematic representation of rDNA with emphasis on Pol I transcription termination features and factors. (B) Sequence of 35S terminator region with designated termination sequences and potential Nrd1 or Nab3 binding sites.

RNA Pol I termination factors



Pol I termination factors:

- DNA-binding protein Reb1
- Pol I subunit Rpa12
- endonuclease Rnt1
- RFB binding protein Fob1
- 5'-3' exonuclease Rat1
+ Rai1 cofactor
(torpedo mechanism)
- RNA helicase Sen1
- DNA-binding protein Nsi1

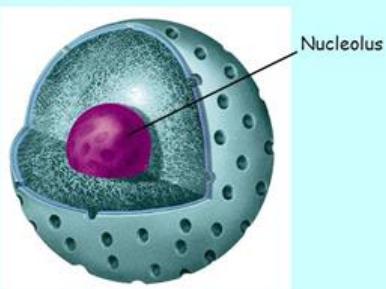


(Reiter et al., EMBO J, 2012; Ha et al., NAR 2012; Merkl et al., MCB 2014)

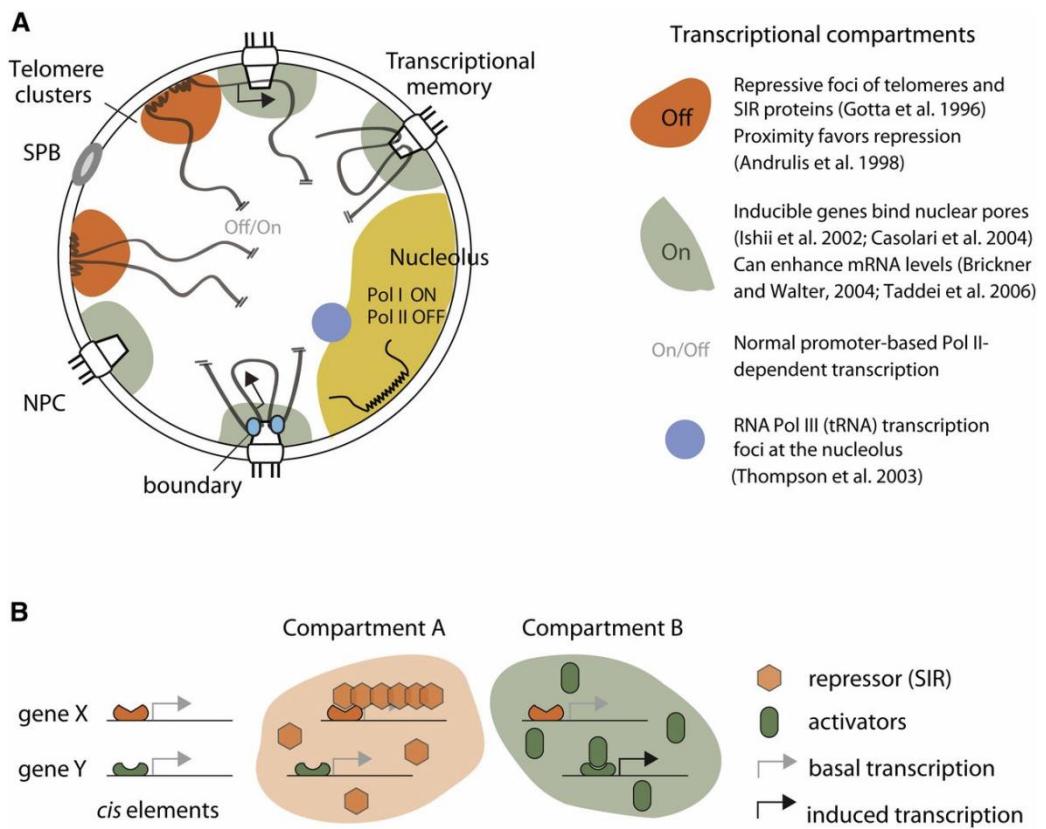
Jąderko – transkrypcja rRNA i początek organizacji rybosomów

NUCLEOLUS

- The nucleolus is where the assembly of ribosomes begins.



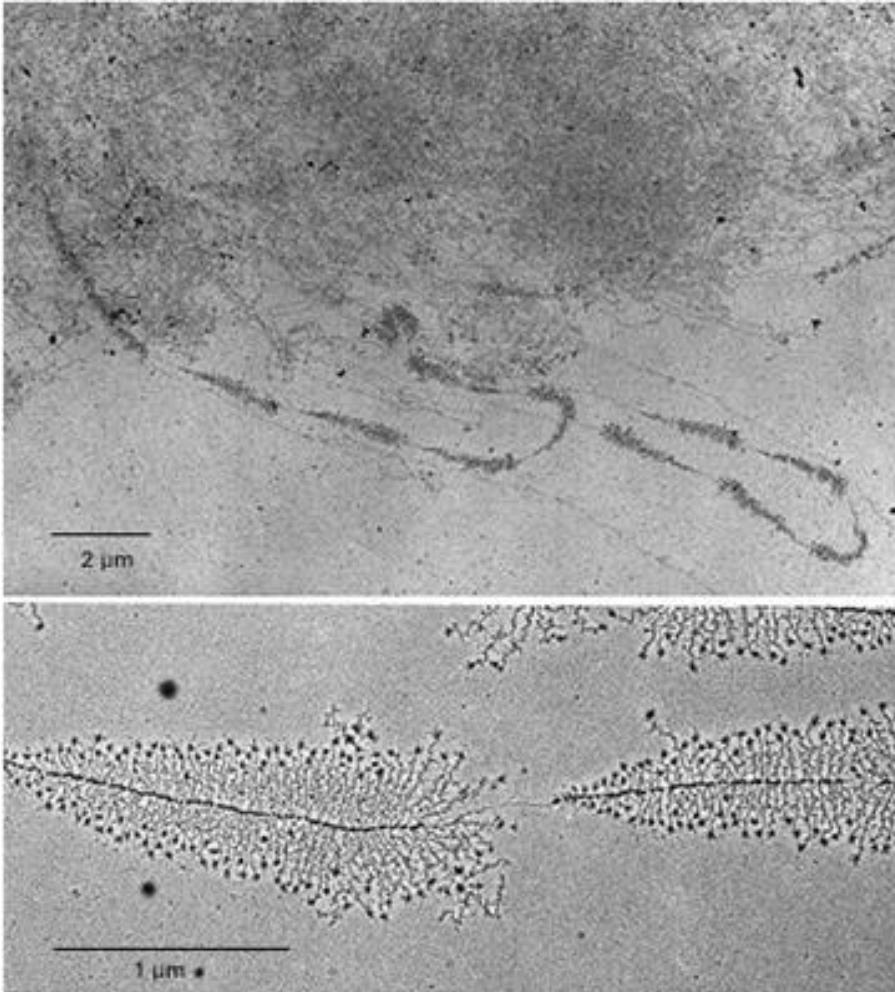
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Angela Taddei and Susan M. Gasser
Genetics, 2012

Geny rDNA zebrane są w jąderku

Miller's spreads – „Christmans tree”

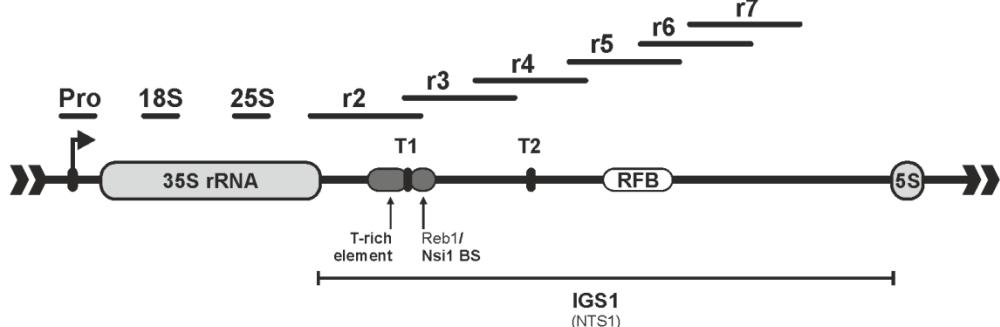


- **Pol I transkrybuje rybosomalny RNA (35S rRNA)**
- **Pol I RNA – ok. 80% całej aktywności transkrypcyjnej jądra komórkowego!!!**
- **U drożdży geny rDNA w 150-200 tandemowych powtórzeniach**

Góra: V.E. Foe, Cold Spring Harbor Symp.
Quant. Biol. 42:723-740, 1978; dół: Ulrich
Scheer, MBC on NCBI.

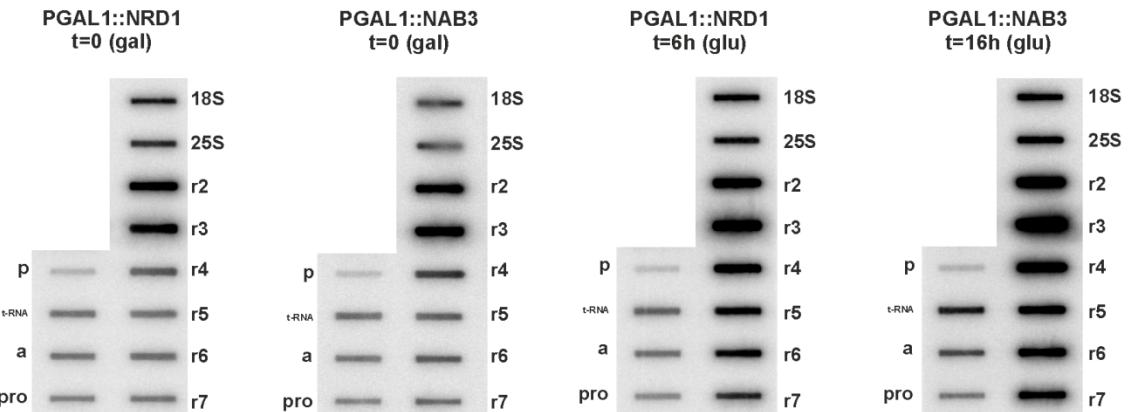
Impaired expression of RNA-binding proteins Nrd1 or Nab3 causes read-through of the Pol I termination region in IGS1

A



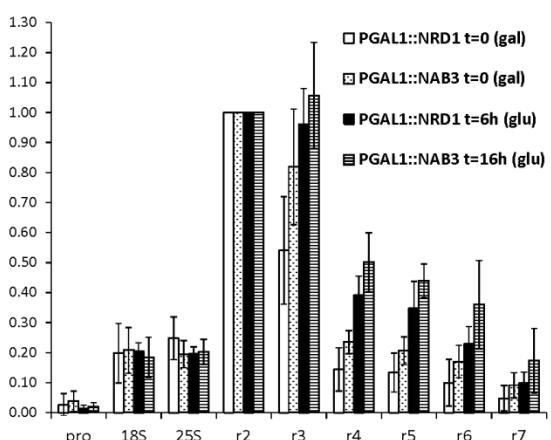
(A) Schematic representation of the positions of TRO probes.

B

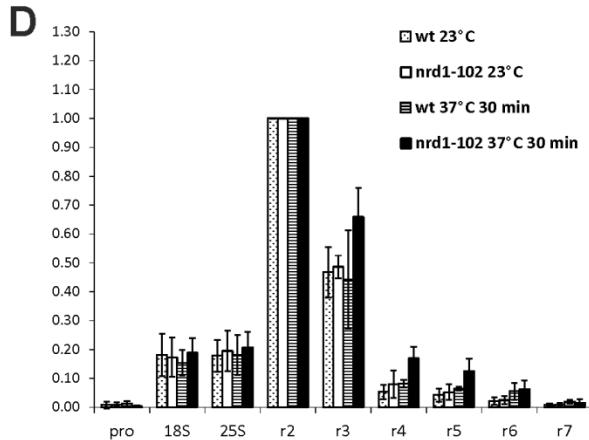


(B) Exemplary TRO hybridizations are shown for the PGAL1::NRD1 and PGAL1::NAB3 strains grown in the galactose-containing (2%) synthetic medium (t=0) or shifted to the glucose-containing (2%) synthetic medium to induce Nrd1 or Nab3 depletion (t=6h or t=16h respectively).

C



D

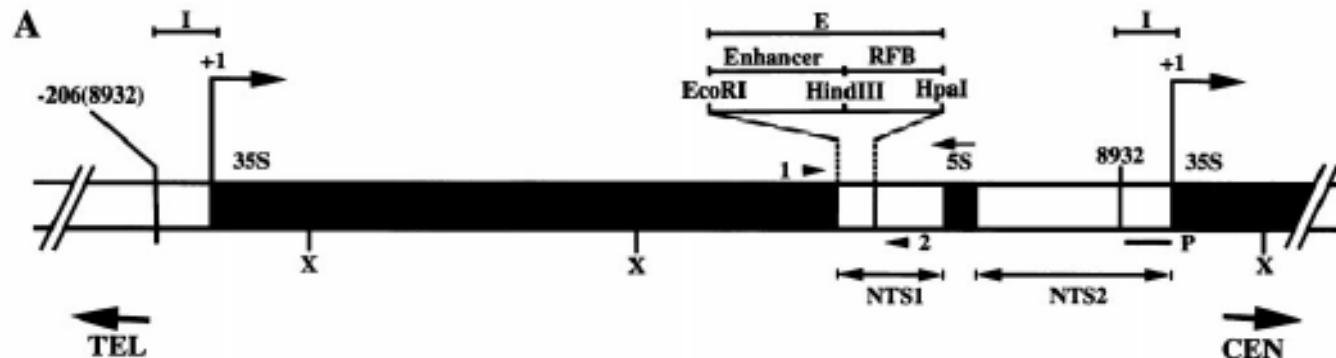


(C) Quantified mean TRO profiles of three independent repetitions of (B) with standard deviation.

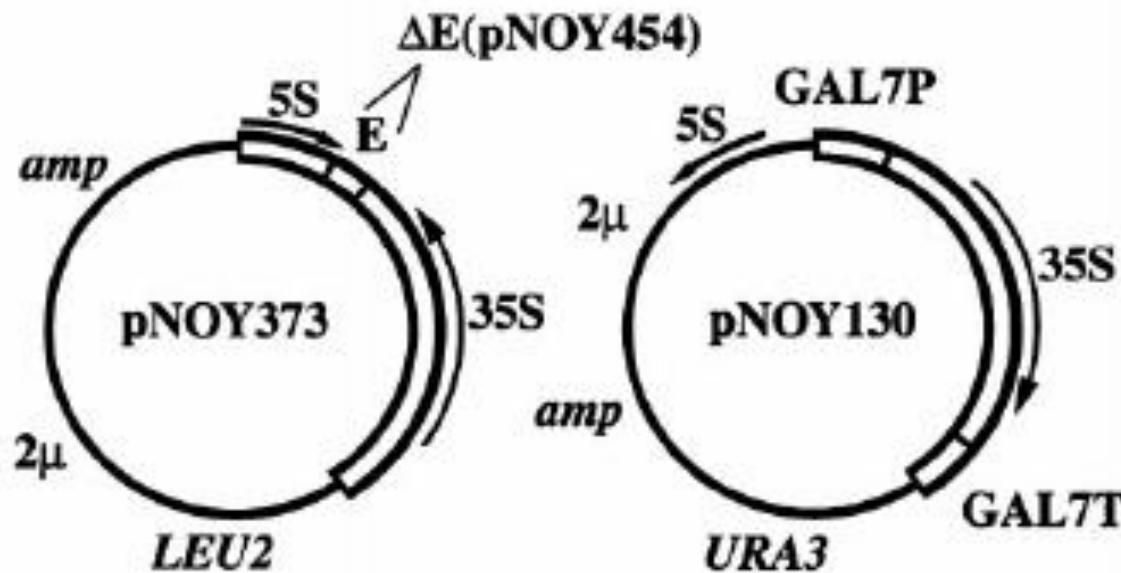
DIRECTED MUTAGENESIS USING *rdnΔ* STRAINS

5542 WAI ET AL.

MOL. CELL. BIOL.



NOY908 = *rdnΔΔ* + pNOY373 *pPOLI*-35S rDNA
(Wei et al., MCB 2001; M. Nomura lab)

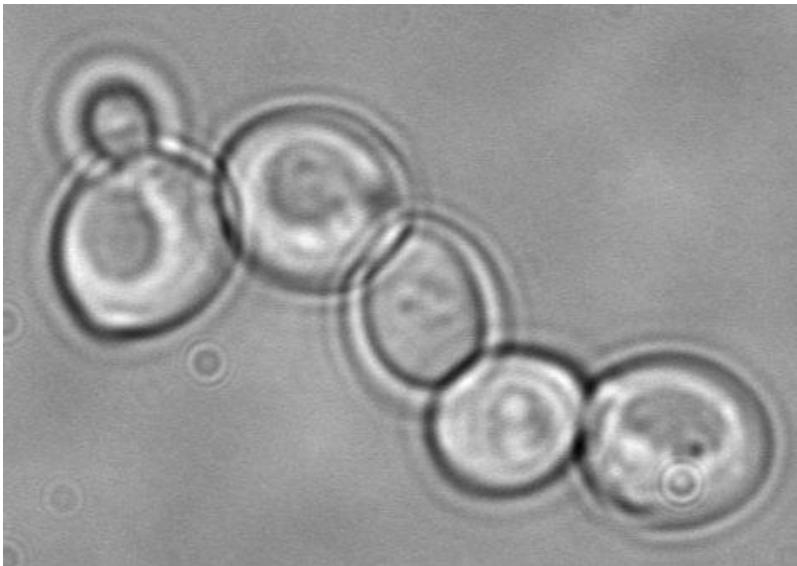


EPISOMAL SYSTEM

We use *Xba*I/*Not*I sites introduced in pNOY373 to exchange ~ 337 bp of *E*-element that covers most important part of *Pol I* terminator for synthetic sequences (synthesized in generay.com) – see also slide 14.

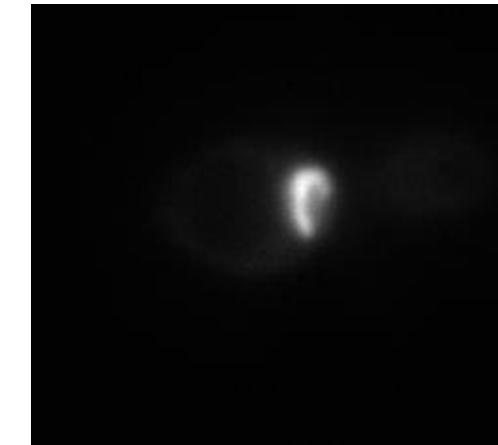
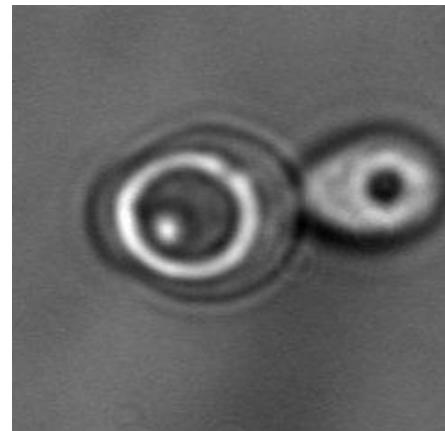
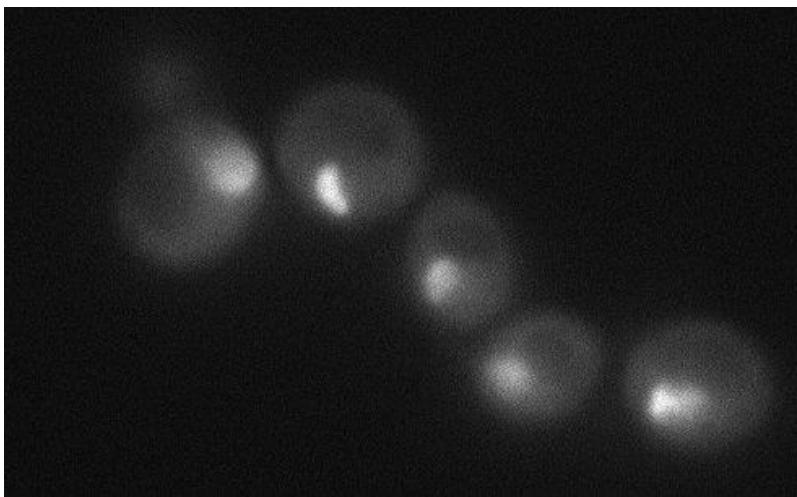
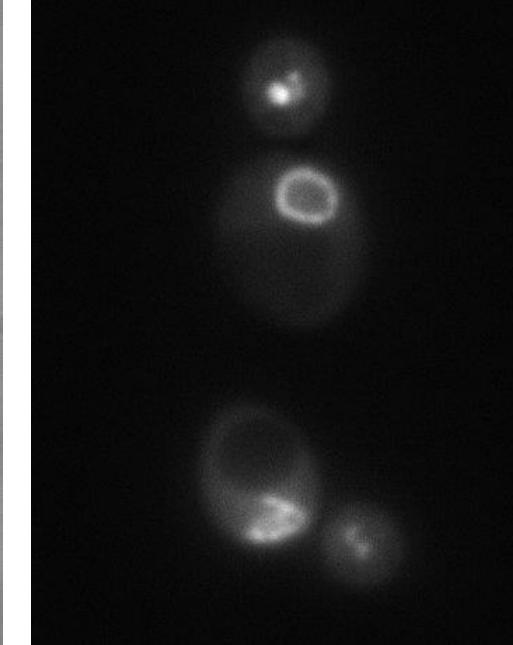
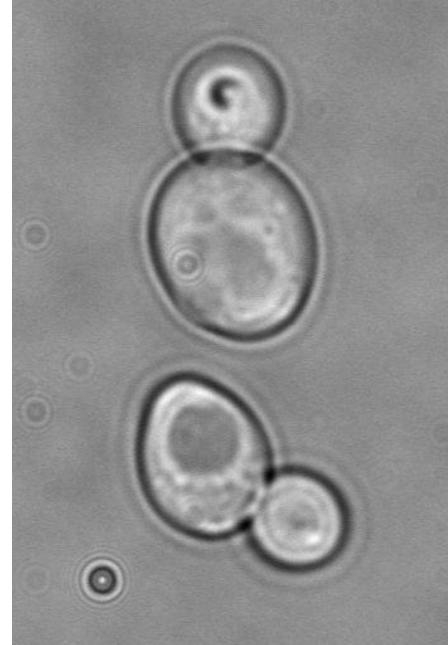
Lokalizacja Nop1-mCherry w szczepie pozbawionym rDNA - zaburzona struktura jąderka

W303



KAY422

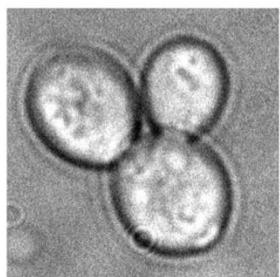
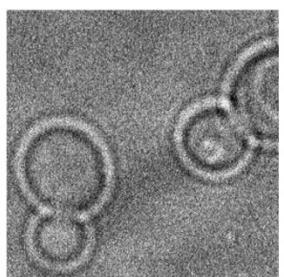
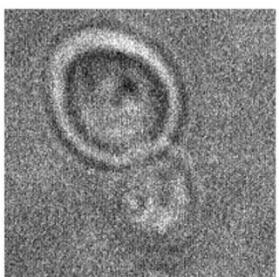
Praca mgr W. Jaworski



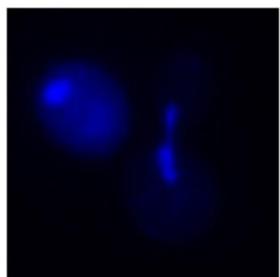
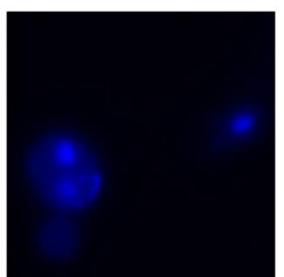
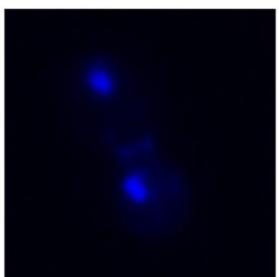
W303a

BY4741

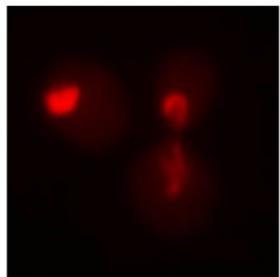
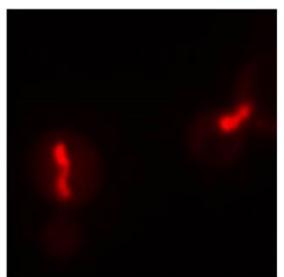
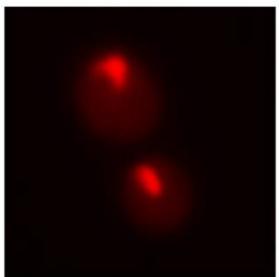
KAY488



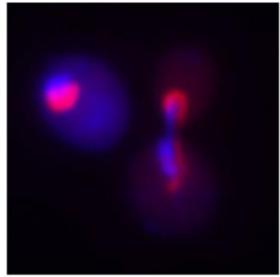
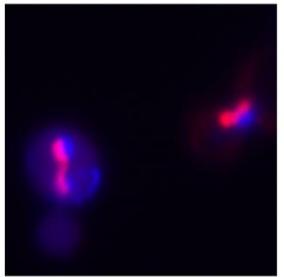
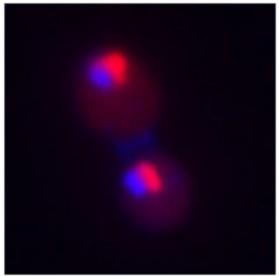
światło przechodzące



Hoechst 33342



mCherry-Nop1



złożenie obrazów

Lokalizacja Nop1-mCherry w szczepie KAY422 pozbawionym rDNA

Widoczna zaburzona struktura jąderka