TRANSPOSITION OF SACCHAROMYCES CEREVISIAE
Ty1 RETROTRANSPOSON DEPENDS ON THE
FUNCTION OF MITOCHONDRIA

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ABSTRACT
Ty1 is a mobile genetic element (transposon) which is transposed spontaneously with a
frequency rate of about 1x10⁻⁷/element/generation. It was found recently that carcinogens
can induce Ty1 transposition to about 1x10⁻⁶/element/generation. While molecular back-
ground of the spontaneous Ty1 transposition is studied in details, the mechanism of car-
cinogen induced Ty1 transposition is not known. We found that mitochondrial functions
participate in Ty1 transposition. The carcinogen induced Ty1 transposition can not take
place in cells with dysfunctional mitochondria (rho⁻ mutants) or cells which fulfill their
energy requirement on glycolysis. Contrary to this, the rate of spontaneous Ty1 transposi-
tion in mitochondrial mutants is increased. The obtained results suggest the existence of
different mechanisms for spontaneous and carcinogen induced transposition of the Ty1
retrotransposon.

Introduction
Saccharomyces cerevisiae cells have mo-
bile genetic elements called Ty, the best
studied of which is Ty1. Similarly to the
known oncoviruses, Ty1 is transcribed into
RNA intermediates which are encapsulated
together with a reverse transcriptase in vi-
rus-like particles (1). After reverse tran-
scription, the new copies of Ty1-DNA are
integrated into new places of the genome.
This integration generates genomic insta-
bility, mutations and large DNA rear-
rangements similar to those found in
mammalian neoplastic cells (2). Thus,
Ty1 is a retrotransposon with a life cycle
and mutagenic properties similar to those
of the oncoviruses and oncogenes.

The frequency rate of spontaneous trans-
position of Ty1 elements is quite low,
about 1x10⁻⁷/element/generation (3). Cer-
tain stress conditions, such as exposure to
UV-light (4), low temperature (5) or nitro-
gen starvation (6) can cause an increase in
transposition activity. It was found recently
that laboratory carcinogens also induce Ty1
transposition to a frequency of about 1x10⁻⁶
/element/generation. The process of car-
cinogen induced Ty1 transposition depends
on the function of RAD9 gene and transit
through G1 phase of the yeast cells life
 cycle (7). RAD9 is the yeast counterpart of
the human tumor-suppressor gene p53,
which functions are: monitoring of genome
integrity and capacity to delay replication
in G1 phase until repair has been com-
pleted (8). Thus, the induction of Ty1
transposition by carcinogens seems to share
common regulatory elements with the early
stages of the neoplastic differentiation of
cells.

Although the molecular background of
the spontaneous Ty1 transposition is rela-
tively well-studied (2), the mechanism of
carcinogen induced Ty1 transposition is not
known. In this communication we provide
evidence that the carcinogen-induced
retrotransposition of Ty1 does not take place in cells with compromised function of mitochondria while the rate of spontaneous Ty1 transposition is enhanced in cells without functional mitochondria. The results obtained suggest the existence of different mechanisms for spontaneous and carcinogen induced transposition of Ty1 retrotransposon.

Materials and Methods
The Ty1 transposition assay
The Ty1 assay was performed as already described (9). *Saccharomyces cerevisiae* strain DG1141ts1 (MAT α ura3-167 his3Δ200:Ty1::HIS3AI ts1) was used. The strain has a Ty1 element marked with the indicator gene HIS3AI (10) which contains the artificial intron AI inserted in antisense orientation relative to HIS3. Each successful transposition of the marked Ty1 gives rise to one histidine prototrophic colony and requires transcription, splicing, reverse transcription and insertion of the resulting Ty1-DNA into new locations of the genome. Histidine prototrophic colonies can not arise by reversion since the indicator HIS3AI gene is inserted in a genome with deleted HIS3 (the his3Δ200). Thus, the number of the HIS+ colonies is a quantitative measure for the transposition rate of the marked Ty1 transposon.

Standard yeast media were prepared as described (11). Median transposition rates were determined and the “fold increase” of Ty1 transposition was calculated. A “fold increase” of two or more is considered as a positive answer of the Ty1 transposition test.

Generation of rho- strains
Strains with mitochondrial mutations (rho-) were generated with ethidium bromide from DG1141ts1 strain (11). Briefly, ethidium bromide was added to a concentration of 10 μg/ml and the cells were incubated at 30 °C with agitation for 24h in YPD medium. Cells were diluted in water and plated on YPD to obtain single colonies. The rho- mutants were selected as cells unable to form colonies on rich medium with glycerol as the sole carbon source (YPG medium). These mutants lack segments of mitochondrial DNA (mtDNA deletions) but maintain the same nuclear markers, and thus are isogenic to the parental wild type strain (11).

Materials
Nutritional media components were from Difco Chem.Co. All tested carcinogenic substances and Dimethylsulfoxide (Me2SO), which was used as a solvent were purchased from Sigma (USA).

Results and Discussion
Increased spontaneous Ty1 transposition in rho- cells
The wild type rho+ and several rho- mutants isolated from strain DG1141ts1 were studied for spontaneous Ty1 transposition with the help of Ty1 test (9). The determination of transposants for the rho+ strain gave a median rate of 2.44±0.11x10⁻⁷ /element/generation, which is in the range of the spontaneous transposition of Ty1 published by other authors (3, 9). When rho- mutants were studied in strictly the same experimental conditions, a 4-fold higher rate of spontaneous transposition of Ty1 was obtained. For the DG1141ts1rho- strain used further in this study, the rate of transposition was 8.98 ± 0.12x10⁻⁷ /element/generation and very similar values were also obtained for the other three rho- derivatives studied. It should be noted that rho+ and rho- derivatives of strain DG1141ts1 are isogenic and hence the observed different rates in the spontaneous Ty1 transposition are due to the presence (rho+) or absence (rho-) of mitochondrial functions in the tester cells.

It is concluded that the loss of mitochondrial functions in the rho- mutants is accompanied by an increase in the frequency of Ty1 spontaneous transposition.

Carcinogen induced Ty1 transposition occurs mainly in rho+ cells
Carcinogen induced Ty1 transposition was
studied by the Ty1 transposition assay (9). In this assay the tester cells were treated for 30 minutes with carcinogens at concentrations permitting high survival rates (about 60% survivals). Table 1 represents the summarized results of several experiments obtained by the study of 10 laboratory carcinogens with the rho+ strain. Tester cells not treated with carcinogen (control) gave about 1.5% spontaneous rho-, which is very similar to the value reported in the literature for the appearance of the spontaneous rho- mutants (12). Eight out of ten studied carcinogens induced only rho+ transposants. The exceptions were tert-butylhydroquinoline that by unknown yet reasons induced 4.6% rho- transposants and tetrahydrofuran inducing 1.1% rho- transposants. The latter value is lower than the rate of spontaneous appearance of rho- mutants (1.5%) and therefore tetrahydrofuran cannot be considered as an activator of carcinogen induced Ty1 transposition in rho- cells.

The absence of successful Ty1 transposition in rho- cells was remarkable since all carcinogenic substances studied (i) did induced Ty1 transposition in rho+ cells with high efficiency and (ii) belong to groups of carcinogens with quite different mechanisms of action. This suggests that the choice of rho+ cells for Ty1 transposition is not due to individual properties but might reflect a common characteristic of studied substances – the carcinogenicity. For this reason, the carcinogen induced Ty1 transposition was studied in details (Table 2) with tester strains of rho+ and rho- phenotypes. A similar trend was observed in both yeast strains in relation to cytotoxicity of carcinogens and rho+ and rho- cells showed comparable values for survivals. Therefore, the observed different responses of rho+ and rho- cells in the Ty1 transposition test (Table 2) can not be explained by the stress conditions (3, 4, 5) generated by different toxicity to cells, and has to be attributed to the functional state of mitochondria. Cells with functional mitochondria (rho+) responded to treatment with carcinogens by a 7 to 15 folds increase of Ty1 transposition, while the cells with compromised mitochondrial function (rho-) remained silent in the Ty1 test and did not induced Ty1 transposition over the control level.

The principal difference between rho+ and rho- cells is that rho- cells can not respire and depend on glycolysis alone for cellular energy requirements. A rho- phenotype can be mimic if rho+ cells are cultivated in media with a nonfermentable carbon source (11). In further experiments, rho+ cells were cultivated in rich medium with glycerol as a single carbon source (YPG) and studied for carcinogen induction of Ty1 transposition. The results obtained showed that Ty1 transposition was not induced by carcinogens in cells culti-
### TABLE 2

Carcinogen induced Ty1 transposition in rho⁺ or rho⁻ strains of DG1141ts1

<table>
<thead>
<tr>
<th>Carcinogen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Concentration (mM)</th>
<th>Phenotype of DG1141ts1</th>
<th>Survival (%)</th>
<th>Median Rate&lt;sup&gt;b&lt;/sup&gt; of Transposition x10⁻⁷</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O (Control)</td>
<td>100</td>
<td>rho⁺</td>
<td>100</td>
<td>2.89 ± 0.21</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rho⁻</td>
<td>100</td>
<td>6.12 ± 0.33</td>
<td>1.00</td>
</tr>
<tr>
<td>EMS 0.080</td>
<td>rho⁺ 48</td>
<td>18.81 ± 1.10</td>
<td>7.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rho⁻ 56</td>
<td>6.10 ± 1.42</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS 0.050</td>
<td>rho⁺ 63</td>
<td>19.68 ± 0.35</td>
<td>8.56</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>rho⁻ 73</td>
<td>8.01 ± 0.41</td>
<td>0.81</td>
<td></td>
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</tr>
<tr>
<td>AC 340.0</td>
<td>rho⁺ 60</td>
<td>22.80 ± 3.51</td>
<td>7.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rho⁻ 75</td>
<td>6.10 ± 1.05</td>
<td>1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC 130.0</td>
<td>rho⁺ 82</td>
<td>23.61 ± 3.41</td>
<td>7.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rho⁻ 77</td>
<td>11.01 ± 2.83</td>
<td>2.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THF 600.0</td>
<td>rho⁺ 66</td>
<td>36.22 ± 1.11</td>
<td>15.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rho⁻ 62</td>
<td>7.20 ± 0.91</td>
<td>1.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM 100.0</td>
<td>rho⁺ 58</td>
<td>24.41 ± 2.10</td>
<td>7.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rho⁻ 62</td>
<td>7.81 ± 1.80</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> EMS – Ethylmetansulfonate; MMS – Methylmetansulfonate; AC - Acetamide; TAC - Tioacetamide; THF – Tetrahydrofuran; DCM – dichloromethane. <sup>b</sup> Average values from 3-5 experiments.

The results obtained evidence that carcinogen induced Ty1 transposition depends on the functional state of mitochondria and cannot take place in cells with compromised function of mitochondria (rho⁻) or cells which fulfill their energy requirement on glycolysis.

Based on similarities between oncoviruses and Ty1 retrotransposon life cycle (2) and on the observation that carcinogens induce Ty1 transposition (7), we proposed recently a new short-term test for detection of carcinogens (9). Results obtained until now show that the Ty1 test responds positively to substances with carcinogenic activity, e.g. an induction of Ty1 transposition takes place in the tester cells, while mutagens without carcinogenic effect do not induce Ty1 transposition. The Ty1 test seems to be very sensitive in the selective detection of carcinogenic activity and responded positively to laboratory carcinogens that are without effect in all other known short-term tests, like Ames, Zimmerman, DEL tests (9, 13). The reasons for the specific response of the Ty1 test to carcinogens are unknown. In this communication, we provide evidence that mitochondrial functions participate in determination the specific response of Ty1 test to carcinogens. We have found that the carcinogen induced Ty1 transposition (e.g. the positive response of Ty1 test) requires functional mitochondria. An increase in the frequency of Ty1 transposition following treatment with carcinogens cannot take place in cells with dysfunctional mitochondria (rho⁻), or...
in cells that have undergo a metabolic switch from respiration to glycolysis.

Mitochondria participate in a variety of cellular functions. Mitochondria not only produce energy but are also involved in intermediary metabolism, homeostasis, synthesis of lipids, amino acids and nucleotides, active transport, cell mobility, cell proliferation, programmed cell death (14). Interestingly, one of the most common and profound features of cancer cells is their defective mitochondrial function (15). Several distinct differences at microscopic, biochemical, metabolic and genetic levels exist between the mitochondria of normal and cancer cells. First, most tumors show mitochondrial dysfunction and are more dependent upon glycolysis rather than oxidative phosphorylation for energy production (16). Second, differential expression of mitochondrial cytochrome oxidase II in benign and malignant tissues has been reported (17). Third, mutations in mitochondrial DNA are also commonly found in a variety of cancers including ovarian, thyroid, salivary, kidney, liver, lung, colon, gastric, brain, bladder, breast cancers and leukemia (15, 17, 18).

Saccharomyces cerevisiae is an excellent model system to study mitochondrial dysfunction. The rho- mutants result from extensive deletion of the mitochondrial genome including the genes coding for complex III (cytochrome b), complex IV and complex V of the respiration chain (14). Since they lack respiratory chain enzymes, the rho- mutants cannot respire but can still grow by using glycolysis as a source of ATP. Mitochondrial respiration is the major endogenous source of reactive oxygen species (ROS) including superoxide \(\text{O}_2^-\), hydrogen peroxide \(\text{H}_2\text{O}_2\) and the hydroxyl radical \(\text{OH}\). ROS generate a variety of DNA lesions that may cause mutations and contribute to a number of degenerative processes including aging and cancer (19). Oxidative DNA damage may also block chromosome replication and promote the appearance of chromosomal loss (20) which is one of the major reasons for the neoplastic differentiation of cells.

The results obtained in our studies on the carcinogen induced Ty1 transposition seem to be well explained by these recent data. In yeast cells many chemical carcinogens primarily attack the mitochondria (21) and this increases mitochondrial ROS production in rho+ cells. ROS are mutagenic (7, 14), a property which induces Ty1 transposition. Recently, it was shown that intracellular levels of ROS decrease after elimination of mitochondrial respiration in rho- cells (14). Therefore, the treatment with carcinogens does not increase the concentration of ROS to mutagenic levels and rho- cells do not induce Ty1 transposition upon treatment with carcinogens. Such explanation is confirmed also by the absence of carcinogen induced Ty1 transposition in rho+ cells cultivated in media with nonfermentable carbon sources since respiration in such cells is switched off and cells are growing on glycolysis.

Although very plausible, this explanation of the results obtained needs further experimental support. In addition, such explanation does not clarify the differential effect of mitochondrial dysfunction on the spontaneous and the carcinogen induced Ty1 transposition. Our results only suggest the existence of different mechanisms of spontaneous and carcinogen induced Ty1 transposition but further studies are needed to ascertain the role of mitochondria in these two processes.

Aknowledgements

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REFERENCES