The ATP Binding Cassette Transporters Pdr5 and Snq2 of Saccharomyces cerevisiae Can Mediate Transport of Steroids in Vivo*

Yannick Mahé‡, Yves Lemoine§, and Karl Kuchler‡‡

From the ‡Department of Molecular Genetics, University and Biocenter of Vienna, A-1030 Vienna, Austria and §Ecole Supérieure de Biotechnologie de Strasbourg, Université Louis Pasteur, Illkirch-Graffenstaden, F-67400 Strasbourg, France

(Received for publication, June 27, 1996)

Multiple or pleiotropic drug resistance in the yeast Saccharomyces cerevisiae can arise from overexpression of the Pdr5 and Snq2 ATP binding cassette multidrug transporters. Expression of Pdr5 and Snq2 is regulated by the two transcription factors Pdr1 and Pdr3, as multidrug-resistant pdr1 and pdr3 gain-of-function mutants overexpress both drug efflux pumps. One such pdr1 mutant allele was previously cloned in a genetic screen by its ability to suppress the squelching toxicity mediated by an estradiol-inducible chimeric VP16-human estrogen receptor (VEO) expressed in yeast (Gilbert, D. M., Heery, D. M., Losson, R., Chambron, P., and Lemoine, Y. (1993) Mol. Cell. Biol. 13, 462–472).

In this study, we demonstrate that relief of estradiol toxicity in yeast cells expressing VEO requires functional PDR5 and SNQ2 genes, since a Δpdr5 Δsnq2 double deletion leads to an increased estradiol toxicity. Furthermore, using URA3 as an estradiol-inducible reporter gene, we show that Pdr5 and Snq2, when overexpressed from high-copy plasmids, can reduce the intracellular concentration of estradiol. In contrast, a Δpdr5 Δsnq2 double deletion mutant accumulates almost 30-fold more intracellular estradiol than the isogenic wild type. Indirect immunofluorescence showed that a pdr1–3 mutant massively overexpresses Pdr5 at the plasma membrane, suggesting that estradiol efflux from the cells occurs across the plasma membrane. Our data demonstrate that Pdr5 and Snq2 can transport steroid substrates in vivo and suggest that steroids and/or related membrane lipids could represent physiological substrates for certain yeast ABC transporters, which are otherwise involved in the development of pleiotropic drug resistance.

Pleiotropic drug resistance (PDR)1 in yeast is a well-documented phenomenon that appears quite similar to P-glycoprotein (Pgp or Mdr1) and MRP-mediated multidrug resistance in mammalian cells (1, 2). For example, two homologous yeast ABC transporter genes, PDR5 (3, 4) and SNQ2 (5), were recently shown to represent functional and structural homologues of mammalian Mdr1 and MRP (6), since their overexpression in yeast is associated with PDR development. Elevated levels of Pdr5 and Snq2 lead to resistance against a variety of structurally unrelated cytotoxic compounds including mycotoxins (3), cycloheximide (7), 4-nitroquinoline N-oxide and sulfometuron methyl (5). However, each transporter mediates resistance to only a distinct subset of drugs, and there is very little overlap in the substrate specificity of Pdr5 and Snq2 (3, 8).

Transcription of both PDR5 and SNQ2 is controlled by the transcription regulatory proteins Pdr1 and Pdr3 (4, 9, 10). Loss-of-function mutations such as Δpdr1 and Δpdr3 deletions result in a dramatic decrease of Pdr5 and Snq2 expression (11) and cause a marked drug hypersensitivity. In turn, several gain-of-function alleles of pdr1 and pdr3 have been isolated by their ability to confer a pleiotropic drug resistance phenotype (12, 13). In such drug-resistant pdr mutants, expression of the drug efflux pumps Pdr5 and Snq2 is dramatically increased (11), and it has been shown that pdr1-mediated cycloheximide resistance requires the presence of functional PDR5 (14).

Notably, another pdr1 mutant allele was previously cloned as a suppressor of the estradiol-induced squelching phenomenon caused by a VP16-estrogen receptor fusion protein (VEO) expressed in yeast (15). Induction by estradiol of such a chimeric protein, which has the hormone-binding and the DNA-binding domain of the human estrogen receptor fused to the acidic activation domain of the viral protein VP16, leads to a toxic transcriptional transactivation known as transcriptional squelching and causes lethality. The squelching phenomenon caused by an activator is thought to be due to its competition for a functional interaction with other limiting yet unknown transcription factors (15). The attempt to isolate such limiting factors from a yeast genomic library surprisingly resulted in the repeated cloning of a pdr1 gain-of-function allele (15). One possible mechanism for the Pdr1-mediated suppression of VEO toxicity would be that Pdr1 itself represents the limiting transcriptional intermediary factor interacting with VEO (15). However, the authors proposed that overexpression of estradiol-specific efflux pumps in the isolated pdr1 mutant would be a more likely explanation for the apparent squelching suppression (15).

In this report, we show by using two different experimental strategies that gene dosage variation of PDR5 and SNQ2, both of which were shown to be targets for the transcription factors Pdr1 and Pdr3 (11), can efficiently modulate the intracellular estradiol concentration. Our results demonstrate that suppression of VEO squelching by a pdr1 gain-of-function allele is the consequence of the overexpression of estradiol-exporting efflux...
pumps such as Pdr5 and Snq2. Furthermore, our results suggest that steroid derivatives or related membrane lipids could represent physiological substrates for the yeast ABC transporters Pdr5 and Snq2.

**EXPERIMENTAL PROCEDURES**

**Media, Culture Conditions, and Spot Tests**—Rich medium (YPD) and synthetic medium (SD), supplemented with auxotrophic components, as well as medium containing 5-FOA (5-fluoroorotic acid) were prepared essentially as described elsewhere (16). Yeast strains to be tested for growth on agar plates containing 5-FOA were grown to exponential phase. Identical volumes of a cell suspension (A600 = 0.025), as well as a 1:10 dilution of the culture, were spotted onto the plates. Estradiol was prepared as a 2 mM stock dissolved in absolute ethanol or freshly in dimethyl sulfoxide.

**Yeast Strains and Transformations**—All S. cerevisiae strains including the parental wild type strain YPH500 (17) used in this study are listed in Table I. Strains harboring Δsgq2::hisG and Δpdr5::hisG deletions were constructed in a one-step gene replacement procedure (18) by transforming the corresponding parental strains with the SacI-SstI Δsgq2::hisG-URA3-hisG and SalI-XbaI Δpdr5::hisG-URA3-hisG fragments obtained from pYM25 and pYM31, respectively. Transformants were grown on minimal plates containing uracil and 5-FOA to select for the desired Δsgq2::hisG and Δpdr5::hisG deletion strains. Correct integration of the deletion constructs and proper looping-out was confirmed by PCR analysis of genomic DNA isolated from several transformants (19).

Construction of strain PL3, in which transcription of the URA3 reporter gene can be induced by estradiol via three estrogen-responsive elements in the promoter (3ERE-URA3), has been described in detail elsewhere (20). Transformations of yeast strains were carried out essentially as described previously (21), except for transformations with alternative Pdr5 or Snq2 pumps. In these strains, the intracellular availability of estradiol should be increased, and, consequently, VEO toxicity should be elevated.

**RESULTS**

**Disruption of PDR5 and SNQ2 Increases VEO-mediated Estradiol Toxicity**—Searching a yeast genomic library for genes that could provide relief of the toxicity mediated by VEO, a hER-VP16 fusion protein, resulted in the repeated cloning of a pdr1 allele (15). Pdr1, a transcriptional regulator that can mediate PDR in yeast, has been shown to regulate the expression of several drug pumps, including the ABC transporters Pdr5 and Snq2 (9, 11). The pdr1 allele cloned by Gilbert et al. (15) was also shown to confer increased resistance to cycloheximide, a known Pdr5 substrate, implying that overexpression of Pdr5 in the pdr1 mutant might actually diminish VEO toxicity.

Therefore, we wanted to test if VEO toxicity on media containing estradiol is increased in strains that do not express functional Pdr5 or Snq2 pumps. In these strains, the intracellular availability of estradiol should be increased, and, consequently, VEO toxicity should be elevated.

The pDG3 plasmid encoding the VEO fusion protein was transformed into isogenic yeast strains in which PDR5, SNQ2, or both genes were deleted. Several fresh transformants were assayed for their growth phenotype on estradiol-containing medium. As shown in Fig. 1, growth of the Δpdr5 Δsnq2 double disruptant was severely reduced on plates containing 10–25 μM estradiol, whereas isogenic strains with single deletions of either PDR5 or SNQ2 did not differ in growth compared to the wild type strain. Increasing the estradiol concentration to 50–100 μM led to a complete cell growth arrest by VEO in all strains examined (data not shown). These results demonstrate that the intracellular estradiol concentration was elevated in the absence of functional PDR5 and SNQ2, which led to an increased activation of the toxic VEO fusion protein. Furthermore, these data suggest that the relief of VEO toxicity by the pdr1 allele cloned by Gilbert et al. (15) is most likely due to the overexpression of estradiol-specific efflux pumps such as Pdr5 and Snq2, preventing the accumulation of estradiol and the induction of VEO toxicity.

**Overexpression of Pdr5 and Snq2 Decreases the Intracellular Availability of Estradiol**—As shown above, the absence of functional PDR5 and SNQ2 genes leads to an enhanced estradiol-mediated VEO toxicity. To show that overexpression of both Pdr5 and Snq2 can reduce intracellular estradiol concentra-

---

**Table I**

| Strain      | Genotype                  | Parental strain | Ref. |
|-------------|---------------------------|-----------------|------|
| PL3         | MATa ura3-1! his3-Δ200 leu2-3! trp1::3ERE-URA3 |                |      |
| YPH500      | MATa ura3-52 his3-Δ200 leu2-3! trp1::Δ39 lys2::801mmb ade2–101c |                |      |
| YKKB-13     | MATa ura3-52 his3-Δ200 leu2-3! trp1::Δ39 lys2::801mmb ade2–101c Δpdr5::TRP1 | YPH500          |      |
| YYM6        | MATa ura3-52 his3-Δ200 leu2-3! trp1::Δ39 lys2::801mmb ade2–101c Δpdr5::TRP1 Δsnq2::hisG | YKKB-13         |      |
| YALA-B1     | MATa ura3-52 his3-3,112 his3-11,115 trp1–1 PDR1 |                | 11   |
| YALA-G4     | MATa ura3-52 his3-3,112 his3-11,115 trp1–1 pdr1–3 |                | 11   |
| YYM7        | MATa ura3-52 his3-3,112 his3-11,115 trp1–1 PDR1 | PL3             |      |
| YYM8        | MATa ura3-52 his3-Δ200 leu2-3! trp1::3ERE-URA3 Δpdr5::hisG Δsnq2::hisG | YYM7            |      |
tions and thereby suppress transcriptional squelching, we used a yeast strain whose growth is essentially estradiol-dependent on a medium lacking uracil (20). In this strain, URA3 serves as a reporter gene for the presence of intracellular estradiol, since transcriptional activation of URA3 is induced by the human estrogen receptor (hER) in an estradiol-dependent manner via three estrogen-responsive elements (3ERE) placed upstream of the URA3 gene. This reporter strain expressing the full-length hER is completely estradiol-dependent for growth on a medium lacking uracil (20). In contrast, its growth is inhibited by 5-FOA, since the URA3 gene product converts 5-FOA to a toxic metabolite.

To test whether or not increased PDR5 and SNQ2 gene dosage can modulate intracellular estradiol concentrations, we transformed the reporter strain containing 3ERE-URA3 and hER with high-copy PDR5 and SNQ2 plasmids, along with the appropriate control plasmids. Serial dilutions of several independent transformants were assayed for growth in the presence or absence of estradiol on selective plates either lacking uracil or containing uracil plus 5-FOA. As shown in Fig. 2A, strains harboring a control plasmid (lanes marked – in Fig. 2) were unable to grow on plates lacking uracil, whereas strains expressing the hER were able to grow on plates lacking uracil only in the presence of estradiol; in the absence of estradiol, these strains also failed to grow (data not shown). Strains expressing hER plus PDR5 or SNQ2 from a high-copy plasmid were unable to grow on media lacking uracil when the estradiol concentration was low (1 nM) compared to the strain containing the empty 2μ vector. At elevated estradiol concentrations, all strains expressing the hER containing empty 2μ vector, 2μ PDR5, or 2μ SNQ2 were able to grow on medium lacking uracil (data not shown). Taken together, these results suggest that intracellular estradiol is effluxed from the cells by the plasma membrane pumps Pdr5 and Snq2.

Analysis of the strains for growth on medium supplemented with uracil and containing 5-FOA, both with and without estradiol, gave similar results (Fig. 2B). In the presence of estradiol, the strain expressing the hER did not grow on 5-FOA, since transcriptional activation of the URA3 gene results in a Ura3-mediated production of a metabolite toxic to yeast cells. Overexpression of SNQ2 appeared to relieve this toxic effect (Fig. 2B), while the PDR5 overexpressing strain failed to grow under the same conditions. However, PDR5 overexpression seemed to affect growth on 5-FOA regardless of the presence of estradiol, because growth of the PDR5 overexpressing strain was slightly inhibited on the control plate lacking estradiol.

To compare the expression levels of Pdr5 and Snq2 in different strains, we performed immunoblot analysis with the set of strains analyzed in Fig. 2 (Fig. 3). Immunoblotting revealed that Pdr5 expression was only slightly increased in the strain harboring the 2μ PDR5 plasmid when compared to the strain with the control vector. In contrast, the 2μ SNQ2 plasmid led to a dramatic Snq2 overexpression when compared to wild type levels. Immunological detection of the plasma membrane ATPase Pma1 served as a control for equal protein loadings in each lane. Thus, the expression levels of Pdr5 and Snq2 were not comparable in these strains. Consequently, the results do not allow for an accurate assessment concerning the individual contribution of Pdr5 and Snq2 to modulating intracellular estradiol levels.

**Major Role of Pdr5 in Modulating the Intracellular Estradiol Concentration**—To determine the individual contributions of PDR5 and SNQ2 to the modulation of intracellular estradiol concentration, we constructed 3ERE-URA3 reporter strains deleted for PDR5, SNQ2, or both ABC transporter genes. The isogenic set of PL3-derived strains were then transformed with YEp90 vector alone and YEp90HEGO (hER) and analyzed for

**Fig. 1. Increased estradiol toxicity mediated by VEO in a Δpdr5 Δsnq2 disruptant.** Exponentially grown cultures of YPH500 (wild type), YKKB-13 (Δpdr5), YYM5 (Δsnq2), and YYM3 (Δpdr5 Δsnq2) expressing the VEO fusion protein were tested for growth on selective plates in the absence (control) or presence (10 nM and 25 nM) of the indicated estradiol concentrations.

**Fig. 2. Overexpression of Pdr5 and Snq2 inhibit estradiol-mediated URA3 induction.** The reporter strain PL3 (3ERE-URA3) carrying YEp90 (–) or YEp90HEGO (hER) was transformed with the empty vector YEpplac181 (2μ vector), the PDR5 overexpressing plasmid pYM40 (2μ PDR5) or the SNQ2 overexpressing plasmid pYM36 (2μ SNQ2). Transformants were analyzed for their estradiol-dependent growth phenotype by spot tests. A, growth phenotypes on plates selecting for plasmid maintenance (control) and on a plate lacking uracil containing estradiol (1 nM E2). B, growth phenotypes on selective 5-FOA plates in the absence (control) or in the presence of estradiol (5 nM E2).

**Fig. 3. Protein levels of Pdr5 and Snq2 in the PL3 reporter strain.** Total cell extracts were prepared from strain PL3 transformed with both YEp90 (–) or YEp90HEGO (hER) and with the empty vector YEpplac181 (2μ vector), the PDR5 overexpressing plasmid pYM40 (2μ PDR5), or the SNQ2 overexpressing plasmid pYM36 (2μ SNQ2). The extracts were analyzed by immunoblotting using polyclonal antibodies against Pdr5 and Snq2. Equivalent protein loading in each lane was verified by immunoblotting with polyclonal anti-Pma1 antibodies (Pma1).
their estradiol-dependent growth phenotype on a medium lacking uracil (Fig. 4A) and on a medium containing 5-FOA (Fig. 4B). As shown in Fig. 4A, a Δpdr5 Δsnq2 double deletion strain expressing the hER was able to grow on medium containing very low levels of estradiol (0.1 nM), reflecting the increased intracellular concentration of estradiol. Disruption of PDR5 apparently had a major effect on the accumulation of estradiol, since a Δpdr5 single disruptive strain was able to grow at 0.5 nM estradiol, whereas the Δsnq2 single disruptant failed to grow at this concentration. Increasing the estradiol concentration led to growth of all strains, and we were unable to find conditions that allowed for growth of the Δsnq2 single disruptant but not the wild type strain (data not shown). Nevertheless, the marked difference in the estrogendependent growth phenotypes of the single disruptants versus the Δpdr5 Δsnq2 double deletion strain indicate a synergistic action of Pdr5 and Snq2 in influencing the intracellular estradiol concentrations. In fact, this is further supported by results obtained from analyzing the estradiol-dependent growth characteristics on 5-FOA-containing medium (Fig. 4B). The Δpdr5 Δsnq2 double-disrupted strain showed a severe growth deficiency at 1 nM estradiol, whereas both single disruptants could grow under the same conditions (Fig. 4B). Although these results show that Pdr5 seems to play a major role in effluxing estradiol from cells, it seems obvious that both ABC transporters appear to contribute to this process.

Synergistic Contribution of Pdr5 and Snq2 to Modulating Intracellular Estradiol Levels—As shown above, deletion of both membrane pumps led to elevated intracellular estradiol, which also resulted in an increased estradiol-dependent modulation of both VEO and the URA3 reporter gene expression. These results suggest that Pdr5 and Snq2 may act as estradiol exporting pumps. To further investigate this idea, we directly quantified the estradiol accumulation by an in vivo estradiol-uptake assay using [3H]estradiol.

As shown in Fig. 5, [3H]estradiol accumulation is increased about 3-fold in a Δpdr5 deletion strain, whereas a Δsnq2 deletion does not significantly change the estradiol accumulation when compared to wild type levels. However, deletion of SNQ2 in a Δpdr5 background dramatically potentiates the estradiol accumulation observed in a Δpdr5 single-deletion strain, leading to almost 10-fold and 30-fold elevated intracellular [3H]estradiol in the Δpdr5 Δsnq2 double disruptant over the Δpdr5 and Δsnq2 single mutants, respectively (Fig. 5). In agreement with the results obtained from assaying the growth phenotypes of the URA3 reporter strains (Fig. 4A), these data show that Pdr5 can modulate the intracellular estradiol levels, presumably by mediating cellular efflux of the steroid. Moreover, these results indicate that Snq2, although it does not appear to have a major influence on estradiol export on its own, contributes to intracellular estradiol modulation in a synergistic manner only in concert with Pdr5.

Pdr5 Is Localized to the Plasma Membrane in pdr1–3 Mutants—We have previously demonstrated that Pdr5 is localized to the plasma membrane in wild type yeast cells (23). In addition, we have shown that pdr1–3 gain-of-function mutants overexpress Pdr5 and Snq2 by about 10-fold over wild type levels (11). However, it has been reported that overexpression of certain yeast proteins can result in mislocalization (28). To test whether the normal Pdr5 localization to the plasma membrane is affected in pdr1–3 mutants, we performed indirect immunofluorescence on both a wild type and a pdr1–3 mutant strain, which, like the pdr1 mutant allele cloned by Gilbert et al. (15), exhibits increased cycloheximide resistance (11, 14).

As shown in Fig. 6, both the wild type and the pdr1–3 strain display a ring-like fluorescence signal typical for plasma membrane localization. However, consistent with a dramatic overexpression of Pdr5, the signal is much more intense in the pdr1–3 mutant strain. These results demonstrate that Pdr5 is not only overexpressed in pdr1–3 mutants, but also that it is correctly localized and targeted to the plasma membrane as observed in the wild type situation. These data suggest that the mechanism of intracellular steroid depletion is likely to be Pdr5-mediated efflux across the plasma membrane to the extracellular space.

**DISCUSSION**

In this report we show that Pdr5 and Snq2, two yeast ABC transporters associated with high-copy-mediated pleiotropic drug resistance are able to modulate intracellular estradiol levels. We took advantage of two different experimental systems allowing for monitoring intracellular estradiol concentrations in vivo. First, a human estrogen receptor-VP16 chimeric fusion protein that leads to cell growth arrest upon induction by estradiol (15); second, a 3ERE-URA3 reporter strain whose growth on a medium lacking uracil is entirely estradiol-de-
estradiol in the plasma membrane. Notably, a G185V mutation in the human P-glycoprotein also leads to a dramatic switch in substrate specificity causing preferential recognition of colchicine (30, 31), whereas wild type Mdr1 mediates transport of a variety of different cytotoxic drugs (1), including bioactive peptides such as the yeast α-factor peptide mating pheromone (32, 33).

In summary, our studies and those carried out by others (29), reveal a new class of substrates, namely steroids and glucocorticoids, for the yeast ABC pumps Pdr5 and Snq2, and confirm earlier suggestions that both transporters have additional functions to their proposed role in cellular detoxification (3). This is exciting, since it uncovers yet another common functional feature between Pdr5, Snq2, and mammalian Mdr1 and MRP, whose overexpression render tumors and cultured cells resistant to a variety of cytotoxic drugs (1). Interestingly, dexamethasone-induced apoptosis in mouse lymphoma cells is linked to overexpression of the mouse mdr1 gene (34). Moreover, human Mdr1, like Pdr5, can also transport steroids, glucocorticoids, and mineralocorticoids (30, 31, 35) both in vitro and in vivo, and it was suggested that the physiological function of P-glycoprotein in the human adrenal cortex might involve the transport and/or targeted secretion of steroid hormones or other membrane lipids (31). In fact, by analogy to PDR5, it was proposed earlier that Pgp or similar ABC transporters in mammalian cells could be controlling glucocorticoid potency and steroid hormone action by cell- and/or substrate-specific efflux of receptor ligands (36). Indeed, a yet unidentified ATP-dependent export system for steroids such as cortisone and dexamethasone has been reported in mammalian L929 cells (37). Like yeast Pdr5 (36), the steroid export system in L929 cells can be inhibited by the immunosuppressive agent FK506. Based on this finding, the authors suggest that similar mechanisms may account for the glucocorticoid efflux both in yeast and in L929 cells (36).

Although a classical steroid hormone signaling pathway as it exists in mammalian cells has not been described in yeast, steroid derivatives must have an important role in yeast. Actually, the most important sterol in yeast, ergosterol, represents the main sterol compound of the plasma membrane (38). Thus, it is tempting to speculate that the yeast Pdr5 and Snq2 ABC transporters or newly identified yeast homologues, which now include Pdr10, Pdr11, Pdr12, and Pdr15 (39, 40), may represent the main sterol compound of the plasma membrane (38). LikeyeastPdr5(36),thesteroidexportssystemexists in mammalian cells has not been described in yeast, steroid derivatives must have an important role in yeast. Actually, the most important sterol in yeast, ergosterol, represents the main sterol compound of the plasma membrane (38). Thus, it is tempting to speculate that the yeast Pdr5 and Snq2 ABC transporters or newly identified yeast homologues, which now include Pdr10, Pdr11, Pdr12, and Pdr15 (39, 40), may represent the main sterol compound of the plasma membrane (38). LikeyeastPdr5(36),thesteroidexportssystemexists
Substrate Specificity of Multidrug Resistance Transporters in Yeast

Data presented in this work as well as work from other laboratories (29) have demonstrated that the yeast ABC transporters Pdr5 and Snq2 can mediate the transmembrane transport of steroids and glucocorticoids in vitro. Ongoing studies in our laboratory attempt to test the idea of a specific involvement of yeast ABC drug efflux pumps in maintaining a certain plasma membrane ergosterol composition. The availability of yeast ABC drug efflux pumps in vivo, thereby playing an important role in regulating both steroid hormone action and response in mammalian cells.

Acknowledgments—We are greatly indebted to P. Chambon and R. Losson from the IGBMC, Strasbourg, France, for generously providing hormone action and response in mammalian cells. Thanks to A. Kralli and K. Yamamoto for communicating results prior to publication. The artwork of R. Kukina are very much appreciated.

REFERENCES

1. Kane, S. E. (1996) in Advances in Drug Research (Testa, B., and Meyer, U. A., eds) Vol. 2, pp. 181–252, Academic Press, San Diego
2. Borst, P., Schinkel, A. H., Smit, J. J., Wagenaar, E., Van, D. L., Smith, A. J., Eijtens, E. W., Baas, P., and Zaman, G. J. (1995) Pharmacol. Ther. 60, 289–299
3. Bissinger, P. H., and Kuchler, K. (1994) J. Biol. Chem. 269, 4180–4186
4. Balzi, E., Wang, M., Leterme, S., Van Dyck, L., and Goffeau, A. (1994) J. Biol. Chem. 269, 2206–2214
5. Servos, J., Haase, E., and Brendel, M. (1995) Mol. Gen. Genet. 246, 214–218
6. Zaman, G. J., Flens, M. J., van Leusden, M. R., de Haas, M., Mulder, H. S., Lankelma, J., Pinedo, H. M., Scheper, R. J., Baas, P., Broxterman, H. J., and Borst, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8822–8826
7. Leppert, G., McDevitt, E., Falco, S. C., Van D. T., Ficke, M. B., and Golin, J. (1993) Genetics 135, 13–20
8. Hirata, D., Yano, K., Miyahara, K., and Miyakawa, T. (1994) Curr. Genet. 25, 285–294
9. Decottignies, A., Lambert, L., Catty, P., Degand, H., Epping, E. A., Maye-Rowley, W. S., Balzi, E., and Goffeau, A. (1995) J. Biol. Chem. 270, 18150–18157
10. Katmzmann, D. J., Burnett, P. E., Golin, J., Maye, Y., and Maye-Rowley, W. S. (1994) Mol. Cell. Biol. 14, 4651–4661
11. Maye, Y., Farle-McDermott, A., Nourani, A., Delahode, A., Lamprecht, A., and Kuchler, K. (1996) Mol. Microbiol. 26, 109–117
12. Balzi, E., Chen, W., Ulaszewski, S., Capiaux, E., and Goffeau, A. (1987) J. Biol. Chem. 262, 16871–16879
13. Subik, J., Ulaszewski, S., and Goffeau, A. (1986) Curr. Genet. 10, 665–70
14. Meyers, S., Schauer, W., Balzi, E., Wagner, M., Goffeau, A., and Golin, J.

(1993) Curr. Genet. 21, 431–436
15. Gilbert, D. M., Heery, D. M., Losson, R., Chambon, P., and Lemoine, Y. (1993) Mol. Cell. Biol. 13, 462–472
16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) in Current Protocols in Molecular Biology, Greene Publishing Associates, New York
17. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
18. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–209
19. Sathe, G. M., O’Brien, S., McLaughlin, M. F., Watson, F., and Livi, G. P. (1991) Nucleic Acids Res. 19, 4775–4781
20. Pierrat, B., Heery, D. M., Lemoine, Y., and Losson, R. (1992) Gene (Amst.) 119, 237–245
21. Klieb, R. J., Harriss, J. V., Sharp, Z. D., and Douglas, M. G. (1983) Gene (Amst.) 25, 333–341
22. Haase, E., Servos, J., and Brendel, M. (1992) Curr. Genet. 21, 319–324
23. Egner, R., Maye, Y., Pandjaitan, B., and Kuchler, K. (1995) Mol. Cell. Biol. 15, 5879–5887
24. Dreyfuss, G., Adam, S. A., and Choi, D. Y. (1984) Mol. Cell. Biol. 4, 415–423
25. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
26. Vieira, A., Elkin, R. G., and Kuchler, K. (1994) in Cell Biology: A Laboratory Handbook (Celsius, J. E., ed) Vol. 2, pp. 314–321, Academic Press, San Diego
27. Pringle, J. R., Adams, A. E. M., Drubin, D. G., and Haarer, B. K. (1991) Methods Enzymol. 194, 655–692
28. Rothman, J. H., Hunter, C. P., Valls, L. A., and Stevens, T. H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3248–3252
29. Kralli, A., Bohen, S. P., and Yamamoto, K. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4701–4705
30. van Kalken, C. K., Broxterman, H. J., Pinedo, H. M., Feller, N., Dekker, H., Lankelma, J., and Giaccone, G. (1993) Br. J. Cancer 67, 284–289
31. Ueda, K., Okamura, N., Hirai, M., Tanigawara, Y., Saeki, T., Kikou, N., Komano, T., and Hori, R. (1992) J. Biol. Chem. 267, 24248–24252
32. Kuchler, K., Goransson, M., Visnawathan, M., and Thorner, J. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 579–592
33. Raymond, M., Gros, P., Whiteway, M., and Thomas, D. Y. (1992) Science 256, 232–234
34. Bourgeois, S., Grud, D. J., Newby, R. F., and Rajah, R. M. (1993) Mol. Endocrinol. 7, 840–851
35. Wolf, D. C., and Horwitz, S. B. (1992) Int. J. Cancer 52, 141–146
36. Kralli, A., and Yamamoto, K. R. (1986) J. Biol. Chem. 271, 17152–17156
37. Gross, S. R., Arnow, L., and Pratt, W. B. (1970) J. Biol. Chem. 245, 103–114
38. Zinser, E., Paltauf, F., and Daum, G. (1993) J. Bacteriol. 175, 2853–2858
39. Egner, R., Maye, Y., Pandjaitan, B., Hetes, B., Lamprecht, A., and Kuchler, K. (1995) in Membrane Protein Transport (Rothman, S., ed) Vol. 2, pp. 57–96, JAI Press Inc., Greenwich, CT
40. Balzi, E., and Goffeau, A. (1995) J. Bacteriol. 177, 71–76
41. Metherall, J. E., Waugh, K., and Li, H. (1996) J. Biol. Chem. 272, 2627–2633
42. Metherall, J. E., Li, H., and Waugh, K. (1996) J. Biol. Chem. 271, 2634–2640
43. van der Bie, B., Bie, F., Ten, H., Knol, P., Komman, M., and Van der Velden, K. T., and Borst, P. (1987) EMBO J. 6, 3352–3353
44. Smith, A. J., Thirnemann, H. J., Roelsdor, B., Wirtz, K. W., van Blitterswijk, W. J., Smitt, J. J., Schinkel, A. H., and Borst, P. (1994) FEBS Lett. 354, 263–266
45. Smit, J. J., Thirnemann, H. J., Oude-Elerferink, R. P. J., Green, A. K., Warnaar, E., van Deemter, L., Mol, C. A. A. M., Ottenhoff, R., van der Lugt, N. M., van Roon, F. W., van der Kalk, M. A., Offerhaus, G. J. A., Berns, A. J. M., and Borst, P. (1993) Cell 75, 451–462
46. Ruets, S., and Gros, P. (1994) Cell 77, 1071–1081