ASC1/RAS2 Suppresses the Growth Defect on Glycerol Caused by the \textit{atp1–2} Mutation in the Yeast \textit{Saccharomyces cerevisiae}*

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The enzyme complex is composed of the F$_1$-ATPase (catalytic sector) and the transmembrane F$_0$ proton channel (embedded in inner membrane) (4–6). Both F$_1$ and F$_0$ are necessary for ATP synthase activity, whereas F$_0$ alone retains the ability to hydrolyze ATP (F$_0$-ATPase) (7). The F$_1$-ATPase consists of five different subunits: a, b, γ, δ, and ε. The minimum unit for F$_1$-ATPase activity resides on α-β-subunit dimer (8). The catalytic center is considered to be in the β-subunit (9, 10), and the α-subunit has been reported to play a role in the formation of the catalytic site with it (11). The α-subunit also assists the assembly of other subunits of the F$_1$-ATPase (12) by acting as a chaperone to assist assembly (13). In yeast, all but three F$_0$ subunits of the enzyme are encoded on nuclear DNA. In order to examine the control mechanism(s) of the F$_0$-α-subunit in the complex assembly and function, we characterized several mutants (14) and isolated extragenic suppressors of mutations in the ATP$_1$ gene. One extragenic suppressor for the point mutant, \textit{atp1–2}, was RAS2, a well known regulator of cell proliferation and signal transduction (15–17). This work reveals for the first time that the cellular growth regulatory activity of RAS2 is linked in some manner with the biogenesis or function of mitochondrial enzyme complexes.

**EXPERIMENTAL PROCEDURES**

\textbf{Yeast Strains— Saccharomyces cerevisiae} DC5 (MATa leu2–3 leu2–112 his3 can1–11) (18) was used as a wild-type strain. Nuclear petite mutants XJY11 (MATa leu2–3 leu2–112 his3 ATP1–1 can1–11) and XJY12 (MATa leu2–3 leu2–112 his3 ATP1–1 can1–11) have been reported previously (18). The mutant allele, \textit{atp1–2}, is the same as the \textit{N9–84} mutation reported previously (14, 18). SKY4010 (MATa leu2–3 leu2–112 his3 \textit{ATP1}::HISS can1–11) was constructed by disruption of the \textit{ATP1} gene in DC5 with a 4.2 kb \textit{EcoRI}-\textit{SpHl} fragment containing \textit{atp1::HISS} allele.

\textit{Escherichia coli} Strains—MC1066 (F $^\prime$ lacPOZ lacY1 galK rpsL hsdR trpC60 leu2–66 proAB F' lac) was used for the preparation of plasmids for yeast genetics. One \textit{Shi}NvF$^\prime$ (Invitrogen, San Diego, CA) and XL1–BLUE MR$^\prime$ KAN (Stratagene, La Jolla, CA) were used for the sequencing.

\textbf{Media—}\textit{E. coli} carrying plasmids were grown in LB (0.5% yeast extract, 1% bactotryptone, 1% NaCl) plus 50 or 125 μg of ampicillin/ml. Yeast strains were grown on YPG (1% yeast extract, 2% bactopeptone, 3% glycerol), YPG (1% yeast extract, 2% bactopeptone, 3% glycerol, 3% ethanol), YPD (0.5% yeast extract, 0.5% bactopeptone, 0.1% (NH$_4$)$_2$SO$_4$, 0.2% KH$_2$PO$_4$, 0.1% MgSO$_4$, 0.8% glucose), or SD (0.67 yeast nitrogen base without amino acids, 2% glucose, and appropriate nutrients). Solid medium contained 2% agar.

\textbf{Gene Library—}The Sau3AI pool of yeast genomic DNA from DC5 was cloned into the \textit{BanHI} site of a vector YEp13 for the construction of yeast genomic library (19).

\textbf{Plasmids—}A plasmid pYCL12–5 (YCp type) was constructed as follows; approximately 2.9 kb of \textit{EcoRI}-\textit{SpHl} fragment having \textit{ATP1} with its 5‘- and 3‘-flanking regions was cloned into a derivative of YCP50 in

Mitochondrial ATP synthase functions as a key enzyme for ATP production in eukaryotic cells (1). The enzyme is controlled in response to the energy demands of the cell (2). Although considerable attention has been given to the central role of mitochondrial ATP production in the initiation of programmed cell death (apoptosis), little is known about the regulation of ATP synthase during its biogenesis and energy transduction or its links to growth regulatory pathways (3).
which the URA3 was disrupted by LEU2. pJ12–5 consists of the episomal plasmid YEp13 containing ATP1 as described previously (18). Plasmids pOSBH20 (centromere type) and pMTYBH20 (episomal type) were constructed as follows: a 1.6-kb XbaI-NdeI fragment carrying ASC1 with its 5′- and 3′-flanking regions was cloned into pOS51 (Ycp type vector, 1.5 kb, LEU2, CEN3, ARS5, TRP1, his3) or into YEp13, respectively. The plasmid YEpAtp1–2 (YEp type) was constructed as follows: a SacI-HindIII fragment of ATP1 region of pdJ12–5 was replaced with the same fragment from the atp1–2 mutant.

**PCR—**PCR was performed according to the procedure for Gene Amp DNA amplification reagent kit (Perkin-Elmer). The PCR primer pairs used were 1 (5′-GGCAAGACGCTGAAACAT-3′) and 2 (5′-ATGCTAAATAACTCC-3′), 3 (5′-CCGTTAGGCTCAAGAAAGT-3′) and 4 (5′-TCATAACGGACAAGCAGT-3′), and 5 (5′-TGGTGATGTTTCTTACTGCG-3′) and 6 (5′-GATTTCAGGTATTTTGCTG-3′). The primers 1 and 6 are located just outside ATP1 coding region. DNA was amplified in a PCR processor (Perkin-Elmer type 480) by using 30 cycles. Yeast whole DNA as templates were prepared by the previously reported method (20). PCR products were cloned into a vector pCRII (Invitrogen, San Diego, CA) or pCR-Script (Stratagene, La Jolla, CA) for sequencing.

**DNA Sequencing—**Nucleotide sequencing was performed by dideoxy chain termination method (21), and autoradiograms were obtained according to the method previously reported (22). In the process of sequencing, we found misreading in the ATP1 sequence previously reported (18). We re-sequenced the wild-type ATP1 by using a genomic clone and used corrected sequence for comparison. The nucleotide sequences described in this article are available from the DDBJ/EBI/GenBank nucleotide sequence data bases under accession numbers D37848 (ATP1), D37849 (atp1–2), D88458 (atp1–1), and D37950 (ASC1/RAS2).

**Suppression of atp1–2 by ASC1/RAS2 in Yeast**—The nucleotide sequence of atp1–2 (Table II) revealed a guanine to adenine change at the second position in codon 291, is strictly conserved in ATP1 genes of other organisms (Table III), and results in an amino acid substitution from Gly to Asp (Asp) that restored growth of the mutants on glycerol (Fig. 1). Thus, these results support that mutations in ATP1-2 subunit genes reported so far. Therefore, these data indicate that both Gly and Thr of the F1-ATPase a-subunit are essential for function of the ATP synthase.

**Characterization of Mutations in atp1–1 and atp1–2—**The nucleotide sequence of atp1–2 (Table II) revealed a guanine to adenine change at the second position in codon 291, is strictly conserved in ATP1 genes of other organisms (Table III), and results in an amino acid substitution from Gly to Asp. Sequence analysis of atp1–1 also revealed a cytosine to thymine at the second position in codon 383 (Table II) generated an amino acid substitution from Thr to Ile. The residue at Thr is also a strictly conserved amino acid residue among all of the ATP synthase a-subunit genes reported so far. Therefore, these data indicate that both Gly and Thr of the F1-ATPase a-subunit are each essential for function of the ATP synthase.

**Isolation of an atp1–2 Suppressor Gene—**Suppressor genes to atp1–2 (Gly → Asp) that restored growth of the mutants on a non-fermentable carbon source were selected as described (see “Experimental Procedures”). From 10,000 Leu+ transformants, five were able to grow on glycerol and were further characterized. Two of the five plasmids exhibited restriction maps different to that of ATP1 but shared a common region. We denoted the gene contained in these fragments as ASC (for g-subunit complementing gene). The ASC1 suppressor of atp1–2 did not restore the growth defect glycerol of atp1–1 (Fig. 1). Thus, these results support that mutations in atp1–2 and atp1–1 may affect different functions or cellular activities of the F1-ATPase a-subunit. ASC1 failed to restore the growth on glycerol of other ATPase subunit mutants including the ATP1 deletion mutant SKY4010 (data not shown). When the

**RESULTS**

**Biochemical Properties of atp1–2 or atp1–1 Mutants—**Yeast strains carrying atp1–2 (XJJ11) and atp1–1 (XJJ12) mutations fail to grow on the non-fermentable carbon source (Fig. 1) and lack ATPase activity (Table I) (14, 18). Both strains, however, were able to synthesize their full length a-subunits, although in reduced amounts (Fig. 2). The amount of mutant a-subunit was approximately 47% of the parental wild type strain DC5 (Table I) (14, 18). The F1-ATPase activity in the atp1–2 mutant was barely measurable above that of ATP1 deletion, SKY4010. Thus, the atp1–2 mutation essentially led to complete loss of ATPase activity (Table I). The enzyme activity of Δatp1 strain, SKY4010, could be restored to 54% of wild-type enzyme activity by introduction of ATP1 on pYCL12–5. The recovery of enzyme activities in strains atp1–2 and atp1–1 transformed with the same plasmid was 43% and 37%, respectively (Table I). The lower ATPase specific activity of the point mutants transformed with the centromeric plasmid pYCL12–5 suggests that the mutant a-subunit protein probably competes with the plasmid-encoded wild type subunits for assembly into the functional enzyme complex.

**Computer Analysis—**Homology searches were calculated by BLAT (basic local alignment search tool) (23) against all data compiled in the NCBI data base (supplied October 10, 1997 by NCBI).

**Complementation Tests—**Growth on a non-fermentable carbon source, glycerol, was examined by incubation on YPGE medium at 30 °C for 3–4 days.

**Preparation of Mitochondria—**Cells were grown in 50 ml of YPDM medium. After 24 h of incubation at 30 °C, cells (2–4 × 10⁷ cells/ml) were harvested and mitochondria were prepared according to the method reported previously (24).

**Determination of F, ATPase Activity—**F, ATPase activity was measured by minor modification of the method of Pullman et al. (25) by following the decrease of the absorbency at 340 nm. The assay mixture consisted of 50 μM of Tris acetate (pH 7.4), 1 μM of MgCl₂, 0.02 μM of NADH, 2 μM of phosphoenolpyruvate, 2 μM of ATP, 13 μg of lactate dehydrogenase, and 16 μg of pyruvate kinase in 1 ml.

**Western Blotting—**Immunodetection of protein was carried out essentially according to the published procedure (26). Isolated mitochondria (0.3 mg of protein) were suspended in solubilization buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8, 1% SDS, 10% sucrose, 1% β-mercaptoethanol), heated at 100 °C for 5 min, and then subjected to SDS-polyacrylamide gel electrophoresis (10% polyacrylamide gel, Tefco Corp., Tokyo, Japan). Western blotting was performed by treatment of gel resolved F1-ATPase subunits, bound anti-F1 a-subunit antibody on the polycylinidene difluoride membranes were assayed mainly by using a Western-Light rabbit kit (Tropix, Inc., Bedford, MA).
**Suppression of atp1–2 by ASC1/RAS2 in Yeast**

**TABLE I**

| Strain  | Allele | Introduced gene (plasmid³) | ATP1 activity | Quantitation of α subunit | Growth on glycerol |
|---------|--------|-----------------------------|--------------|-----------------------------|-------------------|
| DC5     | ATP1   |                             | 1.69 ± 0.36  | 1.00                        | +                 |
| XJY11   | atp1–2 |                              | 0.07 ± 0.00  | 0.47                        | –                 |
| XJY11   | atp1–2 | ATP1 (pYCL12–5)             | 0.72 ± 0.05  | 0.67                        | +                 |
| XJY11   | atp1–2 | ASC1 (pOSBH20)              | 0.08 ± 0.02  | 1.10                        | +                 |
| XJY12   | atp1–1 | ATP1 (pYCL12–5)             | 0.40 ± 0.00  | 0.52                        | –                 |
| XJY12   | atp1–1 | ASC1 (pOSBH20)              | 0.62 ± 0.02  | 0.84                        | +                 |
| XJY12   | atp1–1 | ASC1 (pOSBH20)              | 0.13 ± 0.02  | 0.93                        | –                 |
| SKY4010 | Δatp1  | ATP1 (pYCL12–5)             | 0.07 ± 0.00  | 0                           | –                 |
| SKY4010 | Δatp1  | ASC1 (pOSBH20)              | 0.92 ± 0.07  | 1.00                        | +                 |
| SKY4010 | Δatp1  | ASC1 (pOSBH20)              | 0.07 ± 0.00  | 0                           | –                 |

* Each gene was introduced into each strain using centromeric plasmids.

**TABLE II**

| Strain | Allele   | Nucleotide changes | Codon changes | Amino acid change |
|--------|----------|--------------------|---------------|-------------------|
| XJY11  | atp1–2   | 402C → T           | GTC → GTT     | None              |
|        |          | 576C → T           | GCC → GCT     | None              |
|        |          | 872G → A           | GGT → GAT     | G291D             |
| XJY12  | atp1–1   | 402C → T           | GTC → GTT     | None              |
|        |          | 576C → T           | GCC → GCT     | None              |
|        |          | 1148G → T          | ACC → ATC     | T383I             |

**TABLE III**

| Amino acid position | Gly²⁹¹ | Ala²⁹¹ | Thr³⁸³ |
|---------------------|--------|--------|--------|
| Eukaryote Mitochondria | 38/40 95% | 2²⁹¹ | 5% 39²⁹¹/40 98% |
| Chloroplast | 0/17 0% | 17/17 100% | 17/17 100% |
| Prokaryote Cyanobacteria | 0/4 0% | 4/4 100% | 4/4 100% |
| Others | 16/24 67% | 8²⁹¹ | 33% 24²⁹¹ 100% |

* Chlamydomonas reinhardtii and Physarum polycephalum.

An exceptional organism is evening primrose.

**Mycobacterium leprae**, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma sp.*, *Streptococcus mutans*, and *Rhodobacter capsulatus.*

**FIG. 2** ATPase α-subunit in strains carrying different ATP1 alleles. Wild-type strain DC5 (lane 1), XJY11 carrying atp1–2 (lane 2), XJY12 carrying atp1–1 (lane 3), and SKY4010 carrying Δatp1 (lane 4) are shown. Fifty nanograms of protein from mitochondria of each strain was subjected to Western blotting. The arrow indicates the location of the α-subunit.

**FIG. 3** Growth curves of DC5 and XJY11 transformants with ATP1 or ASC1 in glucose. Symbols are as follows: •, DC5 (wild-type); ○, XJY11 (atp1–2); ▲, XJY11 with ATP1 (s); △, XJY11 with ATP1 (m); ■, XJY11 with ASC1 (s); □, XJY11 with ASC1 (m). Here, s and m indicate single-copy and multicopy plasmid, respectively. Each strain was cultured in 50 ml of YPG with shaking at 30 °C. Cell numbers were counted every 5 h using a counting cell.

**FIG. 4** Preservation of atp1–2 in the same manner as ASC1 (data not shown). Biochemical Properties of the atp1–2 Mutant Transformed with ASC1/RAS2—The presence of the plasmid encoded ASC1/RAS2 in the atp1–2 mutant caused no change in mitochondrial ATPase activity as long as the yeast RAS2 gene obtained from another source suppressed atp1–2 in the same manner as ASC1 (data not shown).
the non-fermentable substrate like glycerol, the F\textsubscript{1}-ATPase activities increased in ATP1–2 almost 6-fold. The level of enzymatic activity in ATP1–2 transformed with ASC1 reached 30% that of the parental strain grown under the same conditions (Table IV). These results suggest ASC1/RAS2 functions as part of the regulatory circuit linked to the control of F\textsubscript{1}-ATPase subunit synthesis on a non-fermentable carbon source.

The amount of F\textsubscript{1}-ATPase α-subunit protein was measured under different growth conditions to determine if increases in activity reflected enzyme content. The amount of Atp1–2p in XJY11 grown on glycerol only increased 1.3-fold following the introduction of ASC1/RAS2 (Table IV) on a centromeric plasmid. The amount of α-subunit increased about 2-fold in both the XJY11 and XJY12 mutants transformed with ASC1/RAS2 on glucose (Table I). This increase in the F\textsubscript{1}-ATPase α-subunit protein occurred when additional copies of ASC1/RAS2 were present. To further confirm the positive regulatory effect of RAS2 on Atp1p, we determined the level of F\textsubscript{1}-α-subunit in a RAS2 deletion mutant. Disruption of ASC1/RAS2 in DC5 decreased the level of Atp1p compared with that of the parental strain (data not shown).

The combination of protein and enzymatic analysis (Tables I and IV) was consistent with a model that additional copies of RAS2 increased the amount of the F\textsubscript{1}-ATPase subunit protein in some fashion. In the case of the mutant containing a partially functional ATP1–2 subunit, the increase in protein likely provides a threshold level of mutant subunit and activity sufficient to support growth on glycerol. If additional RAS2 gene product suppressed ATP1–2 by causing increased levels of the partially active Atp1–2p, then we should restore growth on glycerol of the ATP1–2 mutant by introducing and expressing additional copies of the mutant gene. To test this, additional copies of ATP1–2 were placed into the XJY11 mutant on the multicopy plasmid (pYEa1p–2). Following transformation, the level of Atp1–2p and F\textsubscript{1}-ATPase activity increased and growth was restored on glycerol (Table IV). These data support the model that ASC1/RAS2 controls the amount of α-subunit and ATP synthase activity in mitochondria in response to a non-fermentable carbon source.

### TABLE IV

| Strain | Allele | Medium | Introduced gene | F\textsubscript{1}-ATPase activity | Quantitation of α-subunit |
|--------|--------|--------|-----------------|---------------------------------|--------------------------|
| DC5    | ATP1   | YPG    |                 | 1.29 ± 0.24                    | 1.00                     |
| XJY11  | ATP1–2 | YPG    | ASC1 (pOSBH29)  | 0.39 ± 0.14                    | 0.60                     |
| XJY11  | ATP1–2 | YPG    | atp1–2 (pYEa1p–2) | 0.36 ± 0.08                   | 1.12                     |
| DC5    | ATP1   | YPDM   |                 | 1.69 ± 0.36                    | 1.00                     |
| XJY11  | ATP1–2 | YPDM   |                 | 0.07 ± 0.00                    | 0.47                     |

* A centromeric plasmid.
* A multi-copy plasmid.

### DISCUSSION

In this paper we show that the specific mutant of ATP1, ATP1–2, but not ATP1–1, can be partially suppressed by RAS2, a mediator of signal transduction. Additionally, this is the first report of mutation sites in ATP1 in S. cerevisiae and will allow further examination of the role of this subunit in cell growth and its control. These studies identify an essential residue in α-subunit function at Thr\textsubscript{383} in the active site and another at Gly\textsubscript{291}, which retains partial enzymatic function and can be suppressed by RAS2 in multiple copies (Fig. 5). There are three copies of ATP1 in yeast (39, 40); however, the results of sequence analysis of an extensive number of cloned PCR products indicate that only one mutation site was present in each mutant described here. In the mitochondria from ATP1–2 as well as ATP1–1, the F\textsubscript{1}-ATPase activity was barely measurable, although Western blotting revealed that there were 47% or 52% level of α-subunits, respectively, compared with the level in the wild-type strain. Expression of ATP1 in the same cell with either Atp1–2p or Atp1–1p yielded a lower specific activity than that for ATP1 expression in the absence of the mutants alleles. This indicated competition for assembly between the defective α-subunits, Atp1–2p or Atp1–1p, and the wild type α-subunit expressed from ATP1.

The unusual observation that RAS2 could specifically suppress the ATP1–2 mutation indicates a relationship between a
growth regulatory pathways involving RAS and mitochondrial energy transduction. Analysis of the atp1–2 mutation site revealed that it is not located in the catalytic domain like atp1–1 but at the boundary where subunit-subunit interaction is noted (Fig. 5). All of the information presented in this study is consistent with the a-subunit of atp1–2 exhibiting residual activity that in the presence of multiple copies of RAS2 reaches a threshold sufficient for growth on a mitochondrial-dependent substrate. On the other hand, the presence of additional copies of RAS2 in the atp1–1 mutant, XJY12, does not partially restore F1-ATPase activity or growth on glycerol. This is because the atp1–1 mutation is in the catalytic domain (Fig. 5).

Previous studies have shown that threshold levels of the energy transducing ATPase complex containing as little as 15% of the oligomycin-sensitive ATPase activity of wild type strains is sufficient to support growth on glycerol (41). This is consistent with the model that increasing the level of Atp1–2p and/or enhancing its assembly will yield sufficient energy transducing complex for growth on glycerol. This model for suppression of atp1–2 was confirmed in the experiment in which the gene dosage of atp1–2 was increased using a multicopy plasmid containing atp1–2. The resulting transformants exhibited an increase in F1-ATPase activity and were able to grow on glycerol (Table IV). Thus, increased Atp1–2p due to gene dosage was a necessary condition to support growth on a non-fermentable carbon source. In the XJY12 transformant, the amount of Atp1–1p also increased (Table I) due to ASC1/RAS2. Thus, ASC1/RAS2 appears to be involved in the regulation of a-subunit content in mitochondria. On glucose, the lack of ASC1/RAS2 stimulation of ATPase protein and activity likely reflects the loss of ASC1/RAS2 plasmid due to non-selective growth conditions.

RAS2 has been previously characterized in yeast in earlier studies (16). One phenotype of different alleles of ras2 is the failure to grow on a non-fermentable carbon source, although a firm characterization of the mechanism of this remains open. RAS2 activates adenylyl cyclase, followed by activation of cAMP-dependent protein kinases (42–44). The presence of a cAMP-dependent protein kinase on the inner membrane of mitochondria has been reported (45, 46). More recent work indicates a role for protein kinase activity in the regulation of mitochondrial transcription (47, 48). Dupont et al. also reported that ccs1/ira2, an attenuator gene of RAS1 and RAS2, confers the resistance to inhibitors of the F 0 part of ATP synthase and increased in the ATP synthesis rate in mitochondria (49, 50). This could suggest a role for RAS in the assembly of mitochondrial and nuclear encoded subunits of the complex.

The present study directly links a growth mediator to mitochondrial energy coupling. We demonstrate here that the ASC1/RAS2 stimulates the F1-ATPase in the atp1–2 mutant. This activation allowed growth on glycerol. Studies of the relationship between mitochondrial function and growth regulatory pathways have yet to be convincingly established in detail. However, considerable activity has recently focused on the pathways that link the function of mitochondria and mitochondrial components to the growth regulatory activities with which they are now firmly established. Most recently, Akt, a protein kinase B, which has been shown to act as an anti-apoptotic regulator at many points in the pathway, is itself regulated by phosphorylation (3). So far, RAS2 functions have

**Fig. 5.** Comparison of amino acid sequences between the main central segment of a-subunit in S. cerevisiae, Schizosaccharomyces pombe, and E. coli. The numbered underlines indicate the putative regions from E. coli research as follows: 1, nucleotide-binding domain; 2, important region for subunit-subunit interaction; 3, catalytic domain. The lines over the sequence indicate putative nucleotide-binding domain from S. pombe. Identical amino acid residues are depicted on a shaded background. Dark shaded amino acids indicate the substitutions that can affect enzyme activity. Amino acids in black boxes show the substitutions that lead to loss of function.
been investigated with glucose as a carbon source. It is well established that the RAS2-cAMP pathway is activated by glucose and that RAS2 is necessary to grow on a non-fermentable carbon source. Studies are currently in progress to better understand if these regulatory pathways share common control points and why gene dosage of RAS2 can influence the amount and function of ATPase subunits in mitochondria.

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REFERENCES
1. Cox, G. B., Devenish, R. J., Gibson, P., Howitt, S. M., and Nagley, P. (1992) in Molecular Mechanisms in Bioenergetics (Ernster, L., ed) pp. 303–315, Elsevier, Amsterdam
2. Boyer, P. D. (1993) Biochim. Biophys. Acta 1140, 215–250
3. Green, D. R., and Reed J. C. (1998) Science 281, 1309–1312
4. Putai, M., Nouni, T., and Maeda, M. (1989) Annu. Rev. Biochem. 58, 111–136
5. Gresser, M. J., Myers, J. A., and Boyer, P. D. (1982) J. Biol. Chem. 257, 12030–12035
6. Boyer, P. D. (1987) Biochemistry 26, 8503–8507
7. Yasuda, R., Noji, H., Kinoshita, K., Jr., and Yoshida, M. (1998) Cell 93, 1117–1124
8. Harada, M., Ohta, S., Sato, M., Ito, Y., Kobayashi, Y., Sone, N., Ohta, T., and Kagawa, Y. (1991) Biochim. Biophys. Acta 1056, 279–284
9. Douglas, M. G., Koh, Y., Dockter, M. E., and Schatz, G. (1977) J. Biol. Chem. 252, 8333–8335
10. Duncan, T. M., and Senior, A. E. (1985) J. Biol. Chem. 260, 4901–4907
11. Wise, J. G., Latchney, L. R., and Senior, A. E. (1981) J. Biol. Chem. 256, 10383–10389
12. Yuan, H., and Douglas, M. G. (1992) J. Biol. Chem. 267, 14977–14970
13. Luiz, A. M., Alcomada, A., and Caeza, J. M. (1990) J. Biol. Chem. 265, 7713–7716
14. Tzagoloff, A., Akai, A., and Needleman, R. B. (1975) J. Biol. Chem. 250, 8228–8235
15. Gibbs, J. B., and Marshall, M. S. (1989) Microbiol. Rev. 53, 171–185
16. Broach, J. R., and Deschenes, R. J. (1990) Adv. Cancer Res. 54, 79–139
17. Thevelein, J. M. (1992) A. v. Leeuwenhoek 62, 109–130
18. Takeda, M., Chen, W.-J., Saltzgaber, J., and Douglas, M. G. (1986) J. Biol. Chem. 261, 15126–15133
19. O’Mally, K., Pratt, P., Robertson, J., Lilly, M., and Douglas, M. G. (1982) J. Biol. Chem. 257, 2997–2103
20. Cryer, D. R., Recleshall, R., and Marmur, J. (1975) Methods Cell Biol. 12, 39–44
21. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
22. Mabuchi, T. (1993) Anal. Biochem. 215, 158–159
23. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
24. Daum, G., Böhn, P. C., and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033
25. Pullman, M. E., Penefsky, H. S., Datta, A., and Racker, E. (1960) J. Biol. Chem. 235, 3322–3329
26. Emr, S. D., Vassarotti, A., Garrett, J., Geller, B. L., Takeda, M., and Douglas, M. G. (1986) J. Cell Biol. 102, 523–533
27. Boury, M., Briquet, M., and Goffeau, A. (1983) J. Biol. Chem. 258, 8524–8526
28. Hack, E., and Leaver, C. J. (1983) EMBO J. 2, 1783–1789
29. Mahendra, R., Spottwood, M. R., and Miller, D. L. (1991) Nature 349, 434–438
30. DeFeo-Jones, D., Scolnick, E. M., Keller, R., and Dhar, R. (1983) Nature 306, 707–709
31. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J., and Wigler, M. (1984) Cell 36, 607–612
32. Mortimer, R. K., Contopoulos, C. R., and King, J. S. (1992) Yeast 8, 817–902
33. Felsen, P., Penin, F., Divita, G., Laverge, J.-P., Di Pietro, A., Goody, R. S., and Gautheron, D. C. (1993) Biochemistry 32, 10387–10397
34. Soga, S., Nouni, T., Takeyama, M., Maeda, M., and Futai, M. (1989) Arch. Biochem. Biophys. 268, 643–648
35. Yama, J., and Senior, A. E. (1990) Arch. Biochem. Biophys. 277, 283–289
36. Maggio, M. B., Pagan, J., Parsonage, D., Hatch, D., and Senior, A. E. (1987) J. Biol. Chem. 262, 8891–8894
37. Rao, R., Pagan, J., and Senior, A. E. (1988) J. Biol. Chem. 263, 15957–15963
38. Felsen, P., Maity, L., Conrath, K., and Poutry, M. (1999) Cell 102, 869–873
39. Mogin, O., Okushiba, T., Satoh, T., Koniyoshi, S., Morishita, C., and Ichimura, Y. (1993) J. Biochem. (Tokyo) 117, 607–613
40. Takeda, M., Sato, H., Ohnishi, K., Satoh, T., and Mabuchi, T. (1999) Yeast 15, 873–878
41. Mukhopadhyay, A., Ah, M., and Mueller, D. M. (1994) FEBS Lett. 343, 160–164
42. Tatchell, K., Robinson, L. C., and Breitenbach, M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3785–3789
43. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsutomo, K., and Wigler, M. (1985) Cell 40, 27–36
44. Field, J., Vojtek, A., Ballester, R., Bolger, G., Colicelli, J., Ferguson, K., Gerst, J., Kataoka, T., Michail, T., Powers, S., Riggs, M., Rodgers, L., Wieland, I., Wheland, B., and Wigler, M. (1990) Cell 81, 319–327
45. Riedel, G., Muller, G., and Bandlow, W. (1985) J. Bacteriol. 161, 7–12
46. Muller, G., and Bandlow, W. (1987) Yeast 3, 161–174
47. Chandrasekaran, K., and Jayaraman, J. (1978) FEBS Lett. 87, 52–54
48. MacEntee, C. M., Cantwell, R., Rahman, M. U., and Hudson, A. P. (1993) Mol. Gen. Genet. 241, 213–224
49. Dupont, C. H., Rigoulet, M., Aigle, M., and Guerin, B. (1990) Curr. Genet. 17, 465–472
50. Busseeriu, F., Dupont, C. H., Boy-Marcotte, E., Mallet, L., and Jaqueset, M. (1992) Curr. Genet. 21, 325–329