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Functional Distinctions between IMP Dehydrogenase Genes in Providing Mycophenolate Resistance and Guanine Prototrophy to Yeast*

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IMP dehydrogenase (IMPDH) catalyzes the rate-limiting step in the de novo synthesis of GTP. Yeast with mutations in the transcription elongation machinery are sensitive to inhibitors of this enzyme such as 6-azauracil and mycophenolic acid, at least partly because of their inability to transcriptionally induce IMPDH. To understand the molecular basis of this drug-sensitive phenotype, we have dissected the expression and function of a four-gene family in yeast called IMD1 through IMD4. We show here that these family members are distinct, despite a high degree of amino acid identity between the proteins they encode. Extrachromosomal copies of IMD1, IMD3, or IMD4 could not rescue the drug-sensitive phenotype of IMD2 deletants. When over-expressed, IMD3 or IMD4 weakly compensated for deletion of IMD2. IMD1 is transcriptionally silent and bears critical amino acid substitutions compared with IMD2 that destroy its function, offering strong evidence that it is a pseudogene. The simultaneous deletion of all four IMD genes was lethal unless growth media were supplemented with guanine. This suggests that there are no other essential functions of the IMDPH homologs aside from IMP dehydrogenase activity. Although neither IMD3 nor IMD4 could confer drug resistance to cells lacking IMD2, either alone was sufficient to confer guanine prototrophy. The special function of IMD2 was provided by its ability to be transcriptionally induced and the probable intrinsic drug resistance of its enzymatic activity.

IMP dehydrogenase (IMPDH) catalyzes the rate-limiting step in de novo GTP synthesis. It is an important enzyme whose abundance and activity are correlated with cellular growth rate (1). The enzymology and pharmacology of IMPDH are well understood in mammalian systems because it is a target of antiproliferative drugs. The IMPDH enzymes across phyla show widely varying sensitivities to mycophenolic acid, with $K_i$ values from 7 nM to 18 mM (3, 4). Also, mutations in human IMPDH may be causally involved in some cases of inherited retinitis pigmentosa (5, 6).

It is becoming clear that duplicated genes are a common feature of many genomes. Members of a duplicated gene family may degenerate into nonfunctional pseudogenes or serve as raw material for evolutionary diversity by acquiring novel functions (7). The human and mouse genomes each contain two IMPDH genes: IMPDH type I is constitutively expressed, whereas IMPDH type II is inducible (8). Saccharomyces cerevisiae contains four IMPDH-like genes (IMD1–IMD4; Fig. 1) that display 83–96% amino acid identity between themselves when aligned pairwise. They are closely related to mammalian IMPDHs, showing 58–60% amino acid identity when aligned pairwise with either of the two human proteins. Yeast IMD1 and IMD2 appear to be related by an ancient chromosomal duplication (9, 10). IMD1 may be a pseudogene because it is very close to the telomere, and it contains a frameshifting insertion (Fig. 1; Refs. 9 and 11). It is not known whether IMD1 is transcribed or can yield a full-length protein in vivo as a result of regulated frameshifting. IMD1 and IMD2 are the most closely related IMD pair (96% amino acid identity) and have proven difficult to delete, presumably due to their telomere-proximal location (Ref. 9; Saccharomyces Genome Deletion Project, www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html). However, strains were constructed in which a large deletion of chromosome I resulted in the loss of the promoter of IMD1, the $5'$-untranslated region, and the first 11 codons. This strain and a similar one containing a comparable deletion of part of chromosome VIII and IMD2 are phenotypically normal, suggesting that neither of these is an essential gene (9). Contrasting results were reported for IMD2 by Niedenthal et al. (12), who indicated that IMD2 deletion leads to lethality.

Deletion of IMD3 or IMD4 does not impact viability (13, 14). Although not located near telomeres, this gene pair is also related through an ancient duplication of portions of chromosomes XII and XIII (10, 15). IMD4 is the only yeast IMD that contains an intron (Fig. 1). Given their high sequence homology and the finding that there are four nonessential IMD genes, it seems likely there is functional redundancy among the members of this family, yet the biological and biochemical role of these genes and their gene products in yeast is poorly understood. Whereas redundancy is suggested, there is also specialization between IMD genes, as indicated by the ability of IMD2 to become transcriptionally induced and provide drug resistance (16–18). Although thought to be necessary, it is not known whether induction of IMD2 is sufficient for the acquisition of drug resistance. Some evidence suggests that IMD3 may also be induced (19).

IMPDH has also been implicated in transcription elongation. Initially, it was shown that mutation or deletion of DST1 (also known as PPR2), the gene encoding elongation factor SII (also known as TFIIS), can render cells sensitive to 6-azauracil and...
mycophenolic acid (MPA) (20–23). Both drugs result in the inhibition of IMPDH, which in turn results in a reduction of cellular nucleotide pools (21). Subsequently, mutation of other elements of the yeast elongation machinery has been shown to confer growth sensitivities to MPA and 6-azauracil (24, 25). Transcriptional induction of IMPDH expression has been observed in both mammalian and yeast cells after drug challenge, indicating that nucleotide pool levels are closely monitored in vivo and that their biosynthesis can be transcriptionally regulated (16, 17, 26). In yeast, it is likely that IMD2 contributes the most to this induction. Genetic evidence indicates that an optimally functioning elongation machinery is important for induction of IMD2 (17–19, 27–29). The inability of yeast with mutations in the transcription elongation machinery to induce IMD2 explains, in part, their sensitivity to IMPDH antagonists. There is, however, more than one mechanism by which yeast can become sensitive to 6-azauracil or MPA (17, 18, 30).

Multiple factors are involved in de-repression of IMD2. Promoter elements governing the response have been identified and shown to confer induction upon heterologous genes (16, 17). The proteins responsible for sensing guanine nucleotide levels and de-repressing or activating transcription are unknown. Although the majority of the inductive response appears to be due to IMD2, and deletion of only IMD2 results in 6-azauracil and MPA sensitivity, there is some indication that other members of this gene family are transcriptionally active and may also be up-regulated during nucleotide deprivation (17, 19). However, assessment of individual IMD transcripts by Northern blot and microarray hybridization techniques is confounded by the high degree of relatedness of their mRNAs. An essential tool in dissecting the differential function of the members of this gene family would be a set of single, double, triple, and quadruple deletions, which would enable the examination of each gene’s contribution to IMPDH activity in the cell both before and after induction and the dissection of any functional redundancy.

Here we have constructed such a set of yeast strains and assayed them for growth on rich media and mycophenolic acid. The induction of each member of the IMD family was studied in isolation in triple IMD mutants. The ability of the different IMD genes to rescue the drug-sensitive phenotype was also investigated. The gene family, taken together, is essential, and its loss renders yeast auxotrophic for guanine. By a number of criteria, IMD1 is functionally inert. We show that IMD2 and, to a lesser extent, IMD4 are induced in the presence of these drugs. IMD3 and IMD4 are clearly functional because either is sufficient for guanine prototrophy, and both are distinct from IMD2 because their overexpression by two different means fails to provide drug resistance. The data indicate that IMD2 encodes an inducible and relatively drug-resistant IMPDH.

**MATERIALS AND METHODS**

**Chemicals**

Guanine (Sigma) was dissolved to 50 mM in 1N NaOH, and 250 μl were spread onto 25 ml of solid medium. Mycophenolic acid (Sigma) was dissolved to 15 mg/ml (w/v) in dimethyl sulfoxide and added to molten media before plates were poured. Zymolyase was obtained from US Biological. G418 sulfate (Cellgro) was dissolved in 0.1M HEPES to 200 mg/ml and used at 250 μg/ml. SC and YPD media have been described previously (31).

**Plasmid Construction**

**IMD Expression Vectors**—Single copy and high copy plasmids containing the IMD1–IMD4 genes were constructed as follows. For IMD1, IMD3, and IMD4, each gene was amplified by PCR using Pfu DNA polymerase (Stratagene), cut with XhoI and NotI, and inserted into similarly cut pRS426. The primers used were 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD1, 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD3, and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD4. A similarly constructed IMD2 plasmid has been described previously (18). The respective plasmids (called pIMD1-Pfu, pIMD2-S288C, pIMD3-Pfu, and pIMD4-Pfu) were used as a source of XhoI-NotI fragments to move the IMD genes to the similarly cut CEN plasmid, pRS316 (pIMD1/316, pRS316-IMD2, pIMD3/316, and pIMD4/316). The pIMD1/316 plasmid was mutagenized (Gene Dynamics, Limited Liability Corp.) by deletion of an adenylate residue 1119 bp downstream from the adenylate of the initiating ATG to create pIMD1-dA316. The corresponding XhoI-NotI fragment was removed from this plasmid and inserted into pRS426 to generate the high copy plasmid, pIMD1-dA/426. For expression of the IMD genes from the IMD2 promoter, DNA encoding the open reading frames (ORFs) was digested with SpeI and XhoI after amplification from the respective plasmids described above using 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' (IMD1 and IMD1-dA), 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' (IMD2, IMD3, and IMD4), and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD1 and IMD1-dA, 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD2, 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD3, and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' and 5'-gaattggtggcggcggggtgtgacaggcgaataatgacgccagggagaa-3' for IMD4 and inserted with the IMD2 promoter (cut with NotI and SpeI after amplification from pIMD2-
S288C using 5' -gacctggtggctgtagcggagcatgtaa-3' and 5'-gactagtgggtgacgagtagttgagtgtg-3').

Promoter deletion derivatives were made by amplifying portions of IMD2 from pIMD2-S288C and inserting them into pRS426. pIMD2-GRE, was generated by inserting SpeI and Xhol-cut PCR product made with 5'-gactagtggctgtagcggagcatgtaa-3' and 5'-gactagtgggtgacgagtagttgagtgtg-3'. The second insert was an 1370-bp AvaII fragment from pIMD2-S288C and 5'-gactagtggctgtagcggagcatgtaa-3'. pIMD2-GRE was created by first amplifying IMD2 sequence using 5'-gactagtgggtgacgagtagttgagtgtg-3' and 5'-gactagtggctgtagcggagcatgtaa-3' digestion was used to transfect yeast. The expected DNA sequences were confirmed for all constructs.

**Yeast Strains**

The yeast strains used in these studies are listed in Table I. DNA transformation was performed as described previously (32).DY868 was constructed by transforming a diploid heterozygous IMD2 knockout strain (BY4743-26035; Research Genetics) with the IMD1 gene disruption cassette, selecting for HIS3 transformants and assaying for integration by PCR using a primer complementary to IMD1 and a primer complementary to HIS3. Strain DY871 was generated from DY868 after transformation with the IMD2 disruption cassette, selection on leucine dropout medium, and confirmation of accurate deletion of IMD2 by PCR using a primer complementary to IMD2 and a primer complementary to LEU2. DY872 was generated from DY871 transformed with an IMD4 gene disruption cassette made by PCR using 5'-accaatcattgtagctgtagcggag-aaacatccacaacatgagctaatacgacagggtagatg-3' to amplify URA3 from the plasmid pRS316. DY872 was sporulated in sporulation medium (31), and tetrads were plated and dissected on guanine-containing YPD. Haploids DY873-DY876 were tested for growth on the respective media, and genotypes were confirmed by PCR.

**PCR**

PCR conditions were performed as follows: 95 °C for 6 min; followed by 30 cycles of 95 °C for 45 s, 60 °C for 45 s, and 72 °C for 1 min 30 s; followed by 9 min at 72 °C. The primers used for diagnosing the intact or deleted state of target genes were as follows: IMD1-intact, 5'-gctcagcggagctgtagcggagcagcgtcagtaa-3' and 5'-gactagtggctgtagcggagcatgtaa-3' digestion was used to transfect yeast. The expected DNA sequences were confirmed for all constructs.

**Northern Analysis**

Cells were grown in liquid media, collected in the logarithmic growth phase, washed once with water, and frozen. Total RNA was isolated from thawed cell pellets by hot phenol extraction and quantitated by measuring absorbance at 260 nm. Total RNA (15 μg) was resolved on a 1% (w/v) agarose gel and blotted onto Zeta-probe GT nylon (Bio-Rad). Filters were baked at 80 °C for 2 h and then prehybridized for a minimum of 5 h at 42 °C in 5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate), 5× Denhardt’s solution, 50% (v/v) formamide, 1% (w/v) SDS, and 100 μg/ml salmon sperm DNA. Filters were hybridized under the same conditions with ~10^10 cpmp of ^32P-labeled DNA probe for 15–18 h. The filters were washed twice at 22 °C in 2× SSC/0.1% SDS for 5 min each and twice in 0.2× SSC, 0.1% SDS for 5 min each, followed by two washes in 0.1× SSC. Filters were exposed to Kodak X-OMat film and quantitated with a Fuji BAS1000 imaging system. The BMP1 probe was made using pIMD4/126 as template and the primers 5'-gctcagcggagctgtagcggagcagcgtcagtaa-3' and 5'-gactagtggctgtagcggagcatgtaa-3' digestion was used to transfect yeast. The expected DNA sequences were confirmed for all constructs.
ability to grow on the selective media representing each integrated marker. The genotypes were confirmed by PCR (data not shown). These 15 haploid deletion strains were tested for guanine auxotrophy by assaying for growth on rich medium lacking guanine (Fig. 2). Because the quadruple deletant was unable to grow, we conclude that the family of IMD genes, taken as a group, is essential for yeast viability. In support of the prediction that IMD1 is a pseudogene, the IMD2-IMD3-IMD4 triple deletant also failed to grow, demonstrating that IMD1 is functionally inert in its natural chromosomal context.

In organisms with a salvage synthesis pathway, the loss of de novo synthesis of guanine nucleotides can be bypassed by providing guanine in the growth media. The quadruple mutant, as well as the IMD2-IMD3-IMD4 triple deletant, showed wild type growth when the medium was supplemented with guanine (Fig. 2). Neither adenine nor hypoxanthine could rescue growth of the quadruple deletant or the IMD2-IMD3-IMD4 deletant (data not shown). Supplementation with xanthine, the product of IMPDH activity, rescued growth of both strains (data not shown). Hence, loss of the IMD gene family rendered cells auxotrophic for guanine. IMD2, IMD3, or IMD4 rescued growth in the absence of guanine; i.e. each was sufficient to support guanine prototrophy.

We also tested the ability of each deletant to grow in the presence of MPA (Fig. 3). All strains lacking IMD2 were sensitive to MPA. (Because the quadruple deletant and the IMD2-IMD3-IMD4 triple deletant cannot grow without guanine supplementation, and guanine circumvents the drug treatment, these two strains were not informative in this assay.) No combination of other deletions, including the simultaneous deletion of IMD1, IMD3, and IMD4, generated such a phenotype. This formally proved that IMD2 is not functionally equivalent to either IMD1, IMD3, or IMD4 or even to all three of these IMD genes together.

The availability of all four triple deletants provided an opportunity for us to test the transcriptional induction of each IMD in isolation. Cells were grown in liquid media, and RNA was analyzed by Northern blotting at varying times after drug
Northern blots were probed individually with each of the four IMD genes to ensure that differing hybridization efficiencies did not bias the detection of any family member’s mRNA. IMD2 and, to a small extent, IMD4 were induced by mycophenolic acid treatment (Fig. 4, Probe: IMD2, lanes 7–9 and Probe: IMD4, lanes 4–6, respectively). Transcript derived from IMD1 was undetectable (Fig. 4, Probe: IMD1, lanes 10–12). Levels of IMD3 mRNA remained fairly constant throughout the treatment (Fig. 4, Probe: IMD3, lanes 13–15).

We used the drug-sensitive phenotype of an IMD2 deletion strain as an assay to test IMD function. Plasmids bearing IMD1, IMD2, IMD3, or IMD4 and a “reshifted” version of IMD1, in which a single adenylate residue was deleted to generate a full-length IMD1 reading frame, were introduced into cells. As expected, episomal IMD2 restored growth (Fig. 5B). Interestingly, IMD3 and IMD4 partially rescued growth when expression of these genes was under control of the IMD2 promoter. Neither IMD1 nor frameshifted IMD1 was effective in restoring growth in equivalent experiments. We confirmed by Northern blot analysis that MPA resulted in transcriptional induction of each family member under control of the IMD2 promoter (data not shown). This suggested that part, but not all, of the ability of IMD2 to confer MPA resistance was due to transcriptional induction and a concomitant increase in

**Fig. 2.** Guanine auxotrophy of IMD deletion strains. Cells of each strain (Table I) were grown to saturation in 5 ml of YPD with 0.5 mM guanine and diluted to an initial A600 of 0.01, followed by five consecutive 10-fold dilutions. Five μl of each dilution were spotted onto YPD plates containing 0.5 mM guanine and 10 mM NaOH or 10 mM NaOH (solvent for guanine) alone. Plates were incubated at 30 °C. Yeast strains used were (reading down from the top row) BY4741, DY873, DY874, DY875, DY876, DY877, DY878, DY879, DY880, DY881, DY882, DY883, DY884, DY885, DY886, and DY887.

**Fig. 3.** Mycophenolic acid sensitivity of IMD deletants. Yeast strains were grown overnight in YPD with 0.5 mM guanine and diluted to an A600 of 0.01 in SC. Five μl of this suspension and 10-fold serial dilutions thereof were spotted onto SC containing no drug or mycophenolic acid and incubated at 30 °C. Yeast strains used were those indicated in the Fig. 2 legend.

**Fig. 4.** Inducibility of each IMD. Yeast strains were grown to an A600 of ∼0.5 in YPD with 0.5 mM guanine. Cells were pelleted, washed with water, and resuspended in SC to an A600 of ∼0.5. Mycophenolic acid (15 μg/ml) was added, and RNA was harvested from aliquots of culture at the indicated times. Northern blots were probed with the indicated IMD genes or SED1 as a control for loading. The strains used were (from left to right) BY4741, DY883, DY884, DY885, DY886, and DY887.
Fig. 5. Functional complementation of IMD2 deletion by expression of yeast IMD family members. Yeast strains lacking IMD2 and transformed with an empty vector or IMD genes on a single copy (A and B) or 2 μ plasmids (C) were tested for mycophenolic acid resistance. Strains used (Table I) were (reading down from the top row) DY731, DY928, DY942, DY929, DY930, DY931, DY980, DY948, DY981, DY950, DY970, DY731, DY917, DY385, DY918, DY919, DY963, and DY964. Cells in logarithmic growth were diluted to an A\text{600} of 0.025. Ten μl of this and 4-fold serial dilutions thereof were spotted onto SC medium lacking uracil and containing or lacking 7.5 or 15 μg/ml mycophenolic acid and grown at 30 °C. Strains in B contained plasmids in which the respective IMD ORFs were driven by the IMD2 inducible promoter.

IMPDH enzyme abundance. If so, we reasoned that overexpression of IMD3 or IMD4 by placing these genes with their natural promoters on high copy plasmids might phenocopy the observations seen in Fig. 5B. When present on high copy plasmids, IMD3 and IMD4 again provided partial relief from drug inhibition (Fig. 5C). We conclude that during drug challenge, the IMD2 gene product is both quantitatively and qualitatively unique among the family of IMPDHs in yeast because part of the cellular response involves making more IMPDH1 enzyme, and even when overexpressed, IMD3 and IMD4 were only partially active in providing function.

Prior studies have identified regions of the IMD2 promoter that govern the IMD2 transcriptional response in reporter assays (16, 18). These include a GRE ~300 bp upstream of the transcription start site and a RE surrounding the transcription start site (Fig. 6). To test whether these elements were important for cell growth and drug resistance, we introduced into cells plasmids with derivatives of the IMD2 promoter driving transcription of the IMD2 ORF (Fig. 6). A copy of IMD2 lacking all promoter sequences upstream of the transcription start site did not confer MPA resistance (Fig. 6, pIMD2-GRE,RE). A construct lacking the GRE but containing the RE was virtually inactive (Fig. 6, pIMD2-GRE,RE). A construct deleted for the RE, whose absence de-represses basal levels of IMD2 transcription but does not affect induction, was fully competent to rescue growth (Fig. 6, pIMD2-RE). Hence, the transcriptional control sequences identified in reporter assays demonstrated the expected behavior in the MPA bioassay, confirming the importance of transcriptional induction in the acquisition of drug resistance.

IMD2 and IMD1 differ by only 20 amino acids. Yet “re-shifted” IMD1 was completely inactive in these assays, even when it was overexpressed. This suggested that the putative transcriptional silencing of IMD1 due to its telomere-proximal location is insufficient to explain its inactivity in vivo. Presumably, 1 or more of the 20 amino acid substitutions inactivated the IMD1 protein. We tested this idea using chimeric constructs made by exchanging restriction fragments between the IMD1 and IMD2 ORFs (Fig. 7A). These chimeric reading frames were placed downstream of the IMD2 promoter, introduced into an IMD2 deletant, and tested for their ability to confer MPA sensitivity to transformants. Substituting the first 184 amino acids of IMD1 for those of IMD2 inactivated the ability of IMD2 to provide drug resistance (Fig. 7A, construct 5; Fig. 7B). Substituting the carboxyl-terminal 339 amino acids of IMD2 with those from IMD1 also inactivated IMD2 (Fig. 7A, construct 4). Hence, there are substitutions in both the amino-terminal third and carboxyl terminal two-thirds of IMD1 that contribute to its inactivity. The 184 amino-terminal residues of IMD1 were independently introduced into IMD2 in two segments, as residues 1–106 or residues 107–184 (Fig. 7A, constructs 5 and 6, respectively). Substitution of the amino-terminal 106 amino acids of IMD1 completely inactivated IMD2 (Fig. 7B, construct 5). Exchanging the segment from 107–184 severely compromised its function (Fig. 7B, construct 6). The latter finding indicates there is yet a third change in the IMD1 sequence that negatively impacts its function in this assay. The region from 1–106 contains three amino acid differences between IMD1 and IMD2. At two of these positions, IMD1 varies from the three other IMD genes that have the same amino acid.

We independently engineered both substitutions into the reading frame of IMD2 by site-directed mutagenesis to test whether either was sufficient to inactivate IMD2 (Fig. 7A). The G93D substitution (Fig. 7, A and B, construct 8) completely inactivated IMD2, whereas the T38A change (construct 7) had little effect. Thus, we have identified one of the amino acid changes that accounts for the biological inactivity of IMD1. The reciprocal change (D93G) introduced into IMD1 did not re-activate it (data not shown). Taken together, these data indicate that IMD1 possesses numerous amino acid substitutions that inactivate its ORF.

Recent reports have described point mutations in a human IMPDH that may be causally involved in the inheritance of retinitis pigmentosa (5, 6). One such mutation is a substitution of asparagine for an aspartate that is conserved between human IMPDH1, IMPDH2, and all four yeast IMD genes. This change was predicted to be highly deleterious to IMPDH function (5). The mycophenolic acid sensitivity assay allowed us to test whether the comparable D → N change in yeast IMD2 compromised its function. The D229N change did not reduce the ability of IMD2 to provide guanine auxotrophy (data not shown) or confer MPA resistance to yeast (Fig. 7B).

DISCUSSION

IMPDH enzymology and pharmacology have been studied extensively in mammalian cells, partly because of the effective drug therapies directed against this enzyme and the protein’s relationship to cell growth and proliferation. Prior studies of the basis for drug sensitivity in yeast were complicated because of the presence of four IMPDH homologs. With four IMPDH homologs, the gene family in yeast is the largest observed thus far. The problem of evaluating functional redundancy and sub-
The guanine response element is required for IMD2 to provide drug resistance. Derivatives of the IMD2 promoter driving the IMD2 ORF (top panels) were tested for their ability to provide drug resistance when transformed into a yeast strain lacking the IMD2 gene. Strains DY964, DY929, DY924, DY926, and DY927 (bottom panels, reading down from the top row) were grown in liquid media with or without mycophenolic acid, diluted, and spotted onto solid media as described in the Fig. 2 legend.

![Diagram showing the guanine response element and its effects on drug resistance](image)

functionalization within gene families is one that is becoming increasingly important as organismal genome projects reveal large numbers of duplicated genes (33). Multigene families also complicate the interpretation of data from microarray and other transcriptome monitoring assays.

The molecular genetic tools available in yeast have allowed us to show that the biological function of the members of the yeast IMPDH gene family is not equivalent. We learned the following: 1) as a group, the yeast IMPDH gene family is not equivalent. We learned to show that the biological function of the members of the other transcriptome monitoring assays. Complication of data from microarray and large numbers of duplicated genes (33). Multigene families also functionalization within gene families is one that is becoming transcriptome monitoring assays.

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Loss of all four IMD genes is lethal unless guanine is provided for salvage synthesis. Because growth on guanine appears normal in the absence of all four IMD genes, it seems unlikely that the gene products provide another essential function. Although it is known that yeast possess a functional salvage pathway for synthesis of guanine nucleotides (34, 35), our data are formal proof that salvage synthesis alone is sufficient to sustain cell viability. The relative balance between these pathways is presumably a function of the availability of guanine in the environment.

**IMD1** was suspected to be a pseudogene because of a frameshift in the ORF as well as its telomere-proximal location. It is not unusual for disabled or pseudogene variants of a gene family to reside at telomere-proximal positions (11). One idea is that gene family members at subtelomeric loci may fall in and out of usage, providing diversity and changes in copy number that are evolutionarily important (11). Because frameshifting and variegation in the extent of silencing at telomeres (telomere position effect) are found in yeast, and some disabled ORFs are transcribed (11, 36), it was formally possible that **IMD1** was expressed. In fact, the promoter region is very similar to that of **IMD2** (~90% in the 300 bp upstream of the start codon), including possession of the important guanine response element (16, 18). By a number of criteria, we have demonstrated that **IMD1** is a pseudogene. First, it lacks both of the biological functions possessed by the other **IMD** genes (guanine prototrophy and mycophenolic acid resistance). Secondly, it possesses at least three inactivating amino acid changes. Third, it does not give rise to significant amounts of mRNA, a conclusion that can only be reached after deletion of the other family members because of cross-hybridization between **IMD** sequences. This is consistent with reporter assays showing that the promoter region of **IMD1** is relatively inactive (16).

The ability of mutated **IMD** genes to complement the drug sensitivity of **IMD2** deletants puts us in a position to test whether human IMPDH mutations involved in a form of autosomal dominant retinitis pigmentosa compromise biological function of the cognate yeast enzyme. Bowne et al. (5) showed that a substitution of asparagine for aspartate at position 226 in human IMPDH1 segregated with disease in at least three families and was absent from normal individuals, leading them to conclude that this change is causally involved in disease. However, this residue is not required for the enzymatic activity of *Escherichia coli* or human IMPDH2 in vitro (37, 38). Our data are consistent with this latter finding because we observed wild type levels of complementation by a version of yeast **IMD2** into which the human mutation was engineered. Because the mutation is thought to cause a dominant genetic disease, we speculate that, if causal, the mutant human IMPDH either interferes with wild type molecules or has acquired a new function that is deleterious to the affected retinal cell type. Expression of the mutant form of yeast **IMD2** in wild type yeast did not significantly compromise drug resistance of a wild type **IMD2** (data not shown).

Although the mammalian **IMPDH** type II gene and yeast **IMD2** are both inducible, their disruptions yield distinct phenotypes. Loss of **IMPDH II** does not result in auxotrophy because its loss affects survival even when guanine is provided to **IMD2**.
mice through their drinking water (39). The more complex development of a metazoan may preclude survival when only salvage synthesis of guanine nucleotides is possible. Yet, like the yeast IMD2–IMD3 (or IMD2–IMD4) gene pair, there is evidence that mouse IMPDH I and IMPDH II have non-overlapping biological functions (39, 40). The human IMPDH1 gene shows additional complexity in alternative promoter usage and gives rise to a variety of transcripts in different tissues (40).

Using a set of IMD deletants, we show directly that IMD2 is functionally unique and confirm a report indicating that IMD2 is not essential (9). We extend prior work showing that deletion of IMD2 alone, but not deletion of IMD1, IMD3, or IMD4, conferred mycophenolic acid sensitivity upon yeast (18) and that overexpression of neither IMD3 nor IMD4 conferred strong resistance to this drug in wild type cells (30). Here we observe that overexpression of IMD3 or IMD4 partially rescues the drug sensitivity of IMD2 deletants, leading us to conclude that at least two factors lead to the unique function of IMD2: its change in abundance due to transcriptional induction, and its amino acid differences relative to IMD3 and IMD4. Indeed, a specific promoter element described in reporter assays (16, 18) is necessary for the role of IMD2 in drug resistance. Curiously, this element is present in all four genes, suggesting that it is not sufficient for transcriptional induction in the chromosomal context. Weak but inducible transcription from IMD1 could be elicited when it was plasmid-borne, indicating that its natural proximity to a telomere silences transcription (data not shown). The drug-resistant properties of IMD2 likely result from one or more of the amino acid positions in which IMD2 differs from IMD3 and IMD4. It is known that IMPDH enzymes from different species have sensitivities to MPA that vary over 4 orders of magnitude (3, 4). We speculate that the Imd2 protein is

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**Fig. 7.** Testing of mutant IMD2 derivatives for the ability to provide drug resistance to a strain lacking IMD2. Chimeric and site-directed mutants of IMD2 (A) were transformed into yeast. The resulting strains (Table I) were tested (B) for their ability to grow in the presence and absence of mycophenolic acid as described in the Fig. 2 legend. The strains used were (reading down from the top row) DY970, DY948, DY976, DY977, DY731, DY982, DY983, DY1017, DY1019, and DY985.
intrinsically less sensitive (has a higher $K_I$) to mycophenolic acid than IMD3 or IMD4.

To unequivocally demonstrate which IMD genes were inducible, we generated a collection of triple deletants containing only a single IMD. Whereas the transcript of IMD4 was slightly inducible, that of IMD3 was not (Fig. 4). This contrasts with the observed increase in IMD3 enzyme seen in response to mycophenolic acid using a proteomic approach (19). The difference could be reconciled if IMD4 mRNA was under translational regulation, or if its product showed reduced degradation rates in the presence of drug. From our Northern blots, it appears that IMD3 and IMD4 contribute the most to constitutive levels of IMPDH, whereas IMD2 is responsive to changes in intracellular guanine.

Interestingly, the natural chromosomal copies of IMD3 or IMD4 were sufficient to prevent guanine auxotrophy, providing strong evidence that they are active IMPDH enzymes. These two genes appear redundant in that they are functionally similar, aside from a slight difference in inducibility. Cells with an inducible version of IMD2 whose promoter lacks its GRE are also guanine prototrophs (data not shown). In other words, IMD2 contains the activities observed in IMD3 and IMD4 but has gained (or IMD3 and IMD4 have lost) an additional function, drug resistance. Perhaps *Saccharomyces* contains drug-resistant IMD2 because it shares a common ancestry with *Penicillium brevicompactum*, which makes mycophenolic acid and may encode a relatively resistant IMPDH. Alternatively, the IMD2 enzyme may have evolved into a relatively drug-resistant enzyme after IMD gene duplication and following speciation between the two ascomycetes.

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REFERENCES

1. Jackson, R. C., Weber, G., and Morris, H. P. (1975) *Nature* **256**, 311–313
2. Hedstrom, L. (1999) *Curr. Med. Chem.* **6**, 545–560
3. Snyder, F. F., Lightfoot, T., and Hodges, S. D. (1995) in *Parasite and Pyrimidine Metabolism in Man* (Sahota, A., and Taylor, M., eds) pp. 725–728, Plenum Press, New York
4. Zhang, R., Evans, G., Rotella, F., Westbrook, E., Huberman, E., Joachimiak, A., and Collart, F. R. (1999) *Curr. Med. Chem.* **6**, 537–543
5. Bowne, S. J., Sullivan, L. S., Blanton, S. H., Cepko, C. L., Blackshaw, S., Birch, D. G., Hughbanks-Wheaton, D., Heckenlively, J. R., and Daiger, S. P. (2002) *Hum. Mol. Genet.* **11**, 559–568
6. Kennan, A., Alverne, A., Pafl, A., Humphries, M., McKe, A., Stitt, A., Simpson, D. A., Demtroder, K., Orntoft, T., Ayuso, C., Kenna, P. F., Farrar, G. J., and Humphries, P. (2002) *Hum. Mol. Genet.* **11**, 547–557
7. Prince, V. E., and Pickett, F. R. (2002) *Nat. Rev. Genet.* **3**, 827–837
8. Nagai, M., Natsumeda, Y., and Weber, G. (1992) *Cancer Res.* **52**, 258–261
9. Barton, A., Bussey, H., Storms, R., and Kaback, D. (1997) *Yeast* **13**, 1251–1263
10. Wolfe, K. H., and Shields, D. C. (1997) *Nature* **387**, 708–713
11. Harrison, P., Kumar, A., Lan, N., Echols, N., Snyder, M., and Gerstein, M. (2002) *J. Mol. Biol.* **316**, 409–419
12. Niedenthal, R., Riles, L., Guldener, U., Klein, S., Johnston, M., and Hegemann, J. H. (1999) *Yeast* **15**, 1775–1796
13. Winzeler, K. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Fourny, F., Friend, S. H., Genten, E., Giaever, G., Hegemann, J. H., Jones, T., Laub, M., Liao, H., Liebkindt, J., Lockhart, D. J., Luo, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebschung, C., Revuelta, J. L., Riles, L., Roberts, J. C., Ross-Macdonald, P., Scherens, B., Seigner, M., Sooohai-Mahadeo, S., Ster, G. R. K., Veronneau, S., Voet, M., Volckaert, G., Ward, T. R., Wysocki, R., Yen, G. S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M., and Davis, R. W. (1999) *Science* **285**, 901–906
14. Giaever, G., Chu, A. M., Ni, C., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucean-Danila, A., Anderson, K., Andre, B., Arkin, A. P., Astromoff, A., El Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtis, M., Davis, K., Deutschbauer, A., Entian, K., Flaherty, P., Fourny, F., Garfinkel, D. J., Gerstein, M., Heit, D., Guldener, U., Hegemann, J. H., Hempel, S., Herman, Z., Homanillo, D. F., Kelly, D. E., Kelly, S. L., Ketzer, P., LalBonte, D., Lamb, D. C., Lan, N., Liang, H., Liao, H., Liu, L., Luo, C., Luisser, M., Mai, R., Menard, P., Ou, S. L., Revuelta, J. L., Roberts, C. J., Rose, M., Ross-Macdonald, P., Scherens, B., Schimmack, G., Shaffer, B., Shoemaker, D. D., Sooohai-Mahadeo, S., Storms, R. K., Stratthen, J. N., Valle, G., Voet, M., Volckaert, G., Wang, C., Ward, T. R., Wilheney, J., Winzeler, E. A., Yang, Y., Yen, G., Youngman, E., Yu, K., Bussey, H., Boeke, J. D., Snyder, M., Philippsen, P., Davis, R. W., and Johnston, M. (2002) *Nature* **418**, 874–891
15. Shigehara, N., and Wolfe, K. H. (1999) *Gene* (Amst.) **23**, 8253–8261
16. Eschbar-Henriques, M., and Daignan-Fornier, B. (2001) *J. Biol. Chem.* **276**, 1523–1530
17. Shaw, R. J., and Reines, D. (2000) *Mol. Cell. Biol.* **20**, 7427–7437
18. Shaw, R. J., Wilson, J. L., Smith, K. T., and Reines, D. (2001) *J. Biol. Chem.* **276**, 32905–32916
19. Eschbar-Henriques, M., Balguerie, A., Monrobt, C., Boucherie, H., and Daignan-Fornier, B. (2001) *J. Biol. Chem.* **276**, 32429–32434
20. Hubert, J.-C., Guenvargh, A., Kammerer, B., Exinger, F., Liljelund, P., and Lacroix, F. (1983) *EMBO J.* **2**, 2071–2073
21. Exinger, G., and Lacroix, F. (1999) *Curr. Genet.* **36**, 29–36
22. Archembault, J., Lacroix, F., Ruet, A., and Friesen, J. D. (1999) *Mol. Biol. Cell.* **10**, 4142–4152
23. Nakanihsi, T., Nakuno, A., Nomura, K., Sekimizu, K., and Natori, S. (1992) *J. Biol. Chem.* **267**, 13200–13204
24. Wind, M., and Reines, D. (2000) *Bioessays* **22**, 327–336
25. Reines, D. (2003) *Methods Enzymol.* in press
26. Giese, D. A., Collart, F. R., and Huberman, E. (1991) *Mol. Cell. Biol.* **11**, 5417–5425
27. Squazzo, S. L., Costa, P. J., Nordstrom, D. L., Kimer, K. E., Simic, R., Jennings, M. L., Link, A. J., Arndt, K. M., and Hatzopoulos, E. A. (2002) *EMBO J.* **21**, 1764–1774
28. Lee, S. K., Yu, S. L., Prakash, L., and Prakash, S. (2002) *Cell* **109**, 823–834
29. Jones, B. S., Altmann, D., and Huberman, E. (2001) *Mol. Biol. Cell.* **12**, 901–911
30. Desmoucelles, C., Pinson, B., Saint-Marc, C., and Daignan-Fornier, B. (2002) *J. Biol. Chem.* **277**, 27036–27044
31. Sherman, F. (1991) *Methods Enzymol.* **194**, 3–20
32. Gietz, D., and Lindquist, S. (1986) *Annu. Rev. Genet.* **20**, 1425
33. Gietz, D., Liang, H., and coworker, J. (1986) *Annu. Rev. Genet.* **20**, 1425
34. Gietz, D., and coworker, J. (1986) *Annu. Rev. Genet.* **20**, 1425
Functional Distinctions between IMP Dehydrogenase Genes in Providing Mycophenolate Resistance and Guanine Prototrophy to Yeast
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