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Genetics

Positive regulation in the general amino acid control of *Saccharomyces cerevisiae*

(*aas* mutants/suppressors/epistasis/molecular cloning/gene dosage)

ALAN G. HINNEBUSCH AND GERALD R. FINK*

Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT **Starvation of yeast for a single amino acid leads to derepression of enzymes in many different amino acid biosynthetic pathways. This general control is regulated by several *trans*-acting genes. Mutations in the *TRA3* gene result in constitutive derepression, whereas mutations in *AAS* genes lead to the inability to derepress. We have isolated *aas* mutations as suppressors of the *tra3-1* mutation. Some of these suppressors are alleles of *AAS2* and others define a heretofore unidentified gene, *AAS3*. We have studied the regulatory behavior of strains containing both *aas* and *tra3* mutations and strains containing the cloned *AAS* genes in high copy number. Either *aas1*$^-$ or *aas2*$^-$ in combination with *tra3*$^-$ has the Tra$^-$ phenotype, whereas *aas3*$^-$ in combination with *tra3*$^-$ has the Aas$^-$ phenotype. These interactions suggest that the *AAS1* and *AAS2* products act indirectly to bring about derepression by disabling the repressive effect of *TRA3*, whereas the *AAS3* product functions more directly and is required even in the absence of the *TRA3* function. When present in high copy number, the *AAS3* gene complements mutations in *AAS1* and *AAS2*, whereas *AAS1* and *AAS2* only complement their cognate mutations. Taken together these data suggest that *AAS1* and *AAS2* are negative regulators of *TRA3*, which in turn is a negative regulator of *AAS3*. *AAS3* is a positive regulator, which is required for the general control response. This model of negative and positive interactions is formally identical to those proposed for the regulation of the galactose and phosphatase systems in yeast.**

In the yeast *Saccharomyces cerevisiae* a number of enzymes in different amino acid biosynthetic pathways are under a common control and derepress when any single amino acid becomes limiting. For example, starvation for histidine leads to derepression of enzymes in the histidine, arginine, tryptophan, and lysine pathways. This cross-pathway regulation, known as general amino acid control, affects at least 24 different amino acid biosynthetic enzymes in six different pathways (reviewed in ref. 1). Regulation appears to occur at the level of transcription, because derepression of several of the enzymes subject to the general control has been correlated with corresponding increases in mRNA levels (1–3). *Cis*-acting regulatory mutations have been identified in the 5′ noncoding regions of the *HIS4* (4) and *HIS3* (5) genes, which prevent normal derepression of these genes. These mutations define a recognition site for a positive regulatory factor that mediates general amino acid control. The core of this recognition site, which has the sequence 5′T-G-A-C-T 3′, exists in multiple copy upstream from *HIS1*, *HIS3*, *HIS4*, and *TRP5*, all of which are subject to general control (3, 5, 6).

Trans-acting regulatory mutations that affect general amino acid control have also been identified. One class (*tra*, *cdr*, *Gen*^{c}) leads to constitutive derepression of enzyme levels and identifies negative regulatory elements. A second class (*aas*, *ndr*) fails to derepress during amino acid starvation and identifies positive regulatory elements (reviewed in refs. 1 and 7).

In this communication, we present information concerning the roles of the different regulatory molecules in general amino acid control. These insights arise from a comparison of the properties of a heretofore unidentified regulatory gene, *AAS3*, with those of previously identified *AAS* genes. Mutations in *AAS3* exhibit an interaction with the *tra3-1* mutation that indicates a more direct regulatory role for the *AAS3* product than for the products of the other regulatory genes. This conclusion is further supported by the effects of high copy plasmids containing the cloned *AAS* genes. The combined results of our experiments indicate that the *AAS3* product is the most likely candidate for a regulatory factor that interacts with the positive control sites recently identified in the *HIS3* and *HIS4* promoters.

MATERIALS AND METHODS

Isolation of *aas*$^-$ Mutants. Strains with the *tra3-1* mutation are temperature sensitive for growth at 37°C (Tsm$^-$ phenotype). Spontaneous Tsm$^+$ revertants of DYA175A (*a tra3-1 his1-29*) were isolated by plating cells on YPD agar (8) and incubating at 37°C. Large colonies were isolated and then subjected to the following genetic analyses. Revertants carrying unlinked suppressors and an intact *tra3-1* mutation were identified by meiotic analysis of diploids formed between the revertants and the wild-type strain S288C. Such diploids gave rise to nonsuppressed Tsm$^-$ spores. Unlinked suppressors were shown to segregate as single Mendelian elements ($2^+:2^-$) in crosses with a second *tra3-1* strain. Strains carrying these suppressors were found to have increased sensitivity to amino acid analogs. In addition, strains carrying the suppressors together with a leaky histidine auxotrophy (*his1-29*) were now completely auxotrophic. Both of these phenotypes are characteristic of known *aas* mutants (9).

Cloning of *AAS* Genes. The yeast strains L865 (*α ndr2-1*† *leu2-3 leu2-112*), L867 (*α aas2-2 leu2-3 leu2-112*), and L869 (*a aas3-1 leu2-3 leu2-112*) were each transformed by the method of Hinnen *et al.* (10) with DNA from a wild-type yeast genomic pool carried on the autonomously replicating yeast plasmid YEp13 (11). Leu$^+$ transformants of each strain were replated for single colonies on SD agar (8) and then replica printed to SD agar containing 30 mM 3-aminotriazole and 40 mM leucine to score for Aas$^+$ transformants. (3-Aminotriazole is an inhibitor of the *HIS3* product and causes histidine starvation.) Each isolate was then

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Abbreviation: kb, kilobase(s).

* To whom reprint requests should be addressed.

† Henceforth we refer to *ndr2-1* as *aas1-3*. *ndr2-1* and *ndr1-1* strains were kindly provided by Ralf Hutter.

examined for the stability of the Leu$^+$ phenotype, and plasmids were recovered from each unstable transformant by transformation of *Escherichia coli*, all as described (3).

We integrated plasmids pAH15, pAH16, and pAH21 into their homologous chromosomal sites by transformation of strains L865, L867, and L869, respectively, using linear molecules generated by cleavage at restriction sites unique to the sequences in the cloned DNA inserts. (pAH15 and pAH20 were digested with *Bam*HI and pAH16 was digested with *Nco* I. The *Nco* I site in pAH16 has not been mapped; however, it is known to reside in the inserted DNA because YEp13 is devoid of this site.) Approximately 10% of the transformants were stable for the Leu$^+$ phenotype when grown nonselectively in YPD. Stable transformants carrying the other cloned DNA sequences were obtained by using plasmids constructed with restriction fragments from the cloned inserts and the nonreplicating *URA3*$^+$ plasmid YIp5. In these cases, undigested DNAs were used in transformations, and the recipient strains were *ura3*$^-$.

Each transformant was crossed by a strain of the same genotype as the recipient strain, except of opposite mating type. Tetrad analysis revealed that the plasmid marker (*LEU2* or *URA3*) segregated 2$^+$:2$^-$ in 10/10 sporulated clones derived from each diploid. To examine linkage of the integrated plasmids to the *aas* mutations used in their cloning, we crossed the transformants to wild-type strains, for those cases in which the transformants are Aas$^+$, or to *leu2*$^-$ or *ura3*$^-$ strains (depending on the plasmid marker), in which the transformants are Aas$^-$. In the former case (plasmids pAH16 and pAH20 and subcloned plasmids derived from pAH17 and pAH19) no Aas$^-$ meiotic segregants appeared in 10 tetrads from each diploid. For the pAH15 transformant, which is Aas$^-$, the Leu$^+$ and Aas$^+$ phenotypes segregated in repulsion in 10/10 tetrads. Transformants carrying integrated YIp5 subclones of three other *aas*-complementing plasmids we isolated are Aas$^-$. In each of these cases, the Ura$^+$ and Aas$^+$ phenotypes segregated independently, indicating that the integration site is unlinked to the *aas* mutation that the original cloned sequences complement. These DNA sequences have not been analyzed further.

Mapping of *AAS* Genes. Yeast transformants carrying the integrated plasmid pAH15, pAH16, or pAH20 were crossed to the multiply marked strains CSH83L (*a spol1 ura3 can1 cyh2 ade2 his7 hom3*), CSH85L (*a spol1 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*), and CSH87L (*a spol1 ura3 his2 leu1 lys1 met4 pet8*). Diploids were isolated, and several hundred mitotic clones of each were examined by replica printing. We looked for the appearance of the recessive phenotypes associated with the markers in the multiply marked CSH strains, as suggested in the 2-μm mapping procedure of S. C. Falco and D. Botstein (personal communication). In a diploid formed between a transformant with integrated pAH16 and a *leu2*$^-$ *met1*$^-$ strain, 24/25 Leu$^-$ segregants were also Met$^-$, strongly suggesting that pAH16 integrates on the left arm of XI (12).

RESULTS

Isolation and Characterization of *aas* Mutations That Suppress *tra3-1*. In addition to causing constitutive derepression of the enzymes subject to general amino acid control, the *tra3-1* mutation results in a temperature sensitivity for growth (9). We have identified *aas* mutations by isolating suppressors of this growth defect. The Aas$^-$ suppressors of *tra3-1* fall into two complementation groups. The members of one of the groups fail to complement and are genetically closely linked to the mutation *aas2-1* isolated by Wolfner *et al.* (9). Therefore, these suppressors are alleles of the *AAS2* locus. The other group is complemented by *aas1-1* (9), *aas2-1* (9), and *ndr1-1* (13) and also shows no linkage to any of these mutations. Therefore, we have assigned these mutations to the locus *AAS3*.‡

Table 1. Phenotypes of *aas* mutants

Genotype	Selected as *tra3-1* suppressor	Growth* in presence of L-Leu	Growth* in presence of L-Leu and 3-AT	Growth* in presence of All-Trp	Phenotype of *aas tra3* double mutant†
aas1-1	−	+	−	+	Tra$^-$
aas1-3	−	+	−	+/−	Tra$^-$
aas2-1	−	+	−	+	(Inviable)
aas2-2	+	+	−	++	Tra$^-$
aas2-3	+	+	−	++	Tra$^-$
aas3-1	+	+/−	−	+/−	Aas$^-$
aas3-2	+	+/−	−	+/−	Aas$^-$
arg9	−	+	−	+	Aas$^-$
ndr1-2	−	+	−	+/−	Tra$^-$
tra3-1	NA	+++	+++	+++	NA
wt	NA	+++	++	+++	NA

NA, not applicable.

* Growth was assayed by spotting cell suspensions on agar medium. L-Leucine and 3-aminotriazole (3-AT) concentrations were 40 mM and 30 mM, respectively. All-Trp media is SD and all amino acids at 0.2%, except leucine at 0.4%. Growth was scored as a confluent patch appearing by 1 day (+++), 2 days (++), or 3 days (+) and weak growth by 3 days (+/−) or no growth (−).

† Phenotypes assessed by growth on all three media given here. *aas3*$^-$ epistasis is complete; *tra3*$^-$ epistasis is complete for all alleles except *aas1-3*.

Table 1 summarizes the characteristics of several of the *aas* mutations that suppress *tra3-1* and compares them to *aas* mutations described previously. All of the *aas* mutations cause increased sensitivity to L-leucine (when it is added in excess to a minimal salts/glucose medium). In addition, all of the mutants grow more slowly than wild type on minimal medium supplemented with a mixture of all of the amino acids except tryptophan. The inhibitory effects of amino acid imbalances on growth were noted previously by Niederberger *et al.* (14) for the mutation *ndr1-1*. The results of Table 1 suggest that such effects apply to all *aas* mutations.

The most important observation regarding the mutants is that the *aas3* mutations suppress the constitutive derepression associated with the *tra3-1* mutation, such that *aas3 tra3-1* double mutants have an Aas$^-$ phenotype. This result contrasts with the interaction between the *tra3-1* mutation and mutations in three other *AAS* genes, *AAS1*, *AAS2*, and *NDR1*, in which the double mutants exhibit the constitutive derepression of *tra3-1* strains (Table 1). [One of the *AAS2* alleles, *aas2-1*, isolated on the basis of amino acid analog sensitivity (9) is lethal in combination with *tra3-1*.]

Cloning of *AAS1*, *AAS2*, and *AAS3*. Complementation of the *aas*$^-$ defect (increased sensitivity to amino acid analogs) was used to screen a yeast genomic recombinant DNA pool for the wild-type *AAS* genes. Plasmid DNA was isolated from the yeast transformants by transformation of *E. coli*. Upon retransformation, each of the isolated plasmids was capable of a wild-type level of complementation of the *aas* mutation used in its cloning. Restriction endonuclease analysis of the plasmids suggested that for each of the three *aas* mutations, *aas1-3*, *aas2-2*, and *aas3-1*, complementing plasmids from two different loci with no common restriction fragments had been isolated. To determine which of the cloned fragments represents the wild-

‡ An *aas3*$^-$ strain was described previously (9); however, its Aas$^-$ phenotype is not the result of a single mutation and has not been studied further.

type DNA sequences of the corresponding *aas*$^-$ mutations, we tested each of the cloned fragments for its ability to integrate by homology at the locus of the *aas* mutation used in its cloning (see *Materials and Methods* for details). We found that for each of the three *aas* mutations, only one of the two classes of complementing plasmids that we isolated contains DNA sequences that direct plasmid integration to the site of the cognate *aas* mutation. We believe that these plasmids contain the authentic *AAS* genes. Their restriction enzyme maps are presented in Fig. 1. The other plasmids integrate at unlinked sites in the yeast genome, which apparently complement *aas* mutations only when present in the cell in many copies.

Complementation of *aas* Mutations by *AAS1*, *AAS2*, and *AAS3* Plasmids. Multicopy plasmids containing the *AAS*$^+$ alleles of *AAS1*, *AAS2*, and *AAS3* were tested for their ability to complement mutations in each of these three *AAS* genes. This test was done by transformation of the same *aas*$^-$ *leu2*$^-$ strains used in cloning the *AAS*$^+$ genes (see *Materials and Methods*) with plasmids pAH15 (*AAS1*$^+$), pAH16 (*AAS2*$^+$), and pAH20 (*AAS3*$^+$) and also with the parent plasmid YEp13, which served as a negative control. The complementation data for these transformants are shown in Table 2. pAH15 and pAH16, which carry the *AAS1* and *AAS2* genes, respectively, only give strong

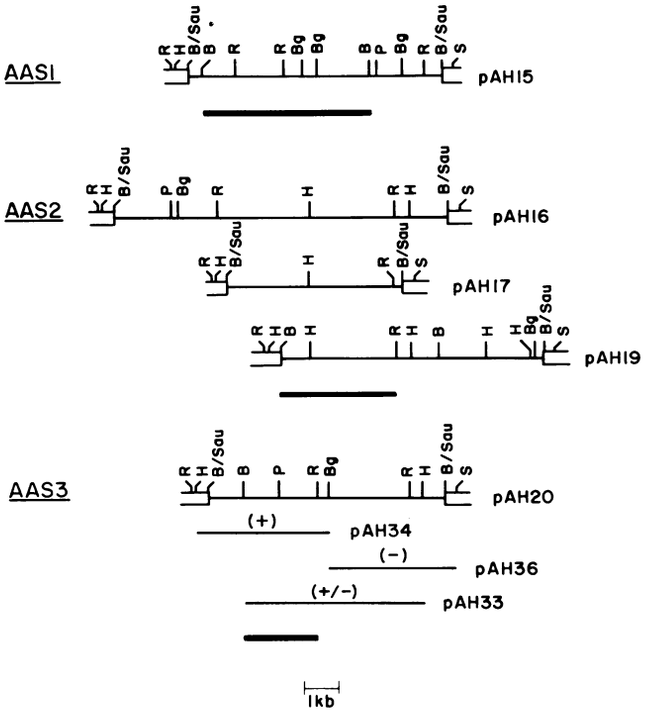

FIG. 1. Maps of *AAS*$^+$ plasmids. The cloned inserts are depicted as thin lines joined to wide, open bars, which represent the YEp13 sequences. The restriction enzyme sites shown are: *Eco*RI (R), *Hin*dIII (H), *Bam*HI (B), *Sau*3A (Sau), and *Bgl* II (Bg). The solid bars depict the fragments used as *AAS* coding sequence probes in the Southern analysis in Fig. 2, based on the following data. For *AAS1*, an integrating plasmid carrying the 4.7-kilobase (kb) *Bam*HI fragment of pAH15 can recombine with an *aas1* mutation to produce *AAS1*$^+$. In the case of *AAS2*, we inferred the position of the gene by comparing the restriction map of pAH16 with those of plasmids pAH17 and pAH19, which were also isolated by complementation of *aas2-2* and carry DNA sequences that direct a nonreplicating plasmid to recombine at *AAS2*. To localize the *AAS3* gene, we tested a set of three overlapping restriction fragments from pAH20 for complementation of the *aas3-1* mutation, after subcloning each fragment into an autonomously replicating plasmid. The subcloned fragments are shown as thin lines below the pAH20 map, and the complementation of *aas3-1* by plasmids carrying these segments is given in parentheses.

Table 2. Complementation of *aas* mutations by cloned *AAS*$^+$ genes on autonomously replicating plasmids

Plasmid	Plasmid-borne *AAS* gene	Complementation*		
		aas1-2	*aas2-2*	*aas3-1*
pAH15	*AAS1*	++++	+	−
pAH16	*AAS2*	+	++++	−
pAH20	*AAS3*	+++	+++	++++
YEp13	None	−	−	−

*Leu$^+$ transformants were scored for complementation by replica-printing patches grown on SD to SD with 30 mM 3-aminotriazole/40 mM leucine. A wild-type control (S288C) was scored ++++ and gave this response after 36 hr; +++ represents the same amount of growth by 2 days; and + corresponds to weak growth after 7 days.

complementation of their cognate mutations. In contrast, pAH20, which carries *AAS3*, complements mutations in *AAS1* and *AAS2* nearly as well as it complements *aas3* mutations.

Each *AAS* Gene Is Unique. One explanation for the ability of the cloned *AAS3* gene to complement multiple *aas* mutations is that the *AAS* genes belong to a set of repeated homologous genes whose products carry out similar functions. We tested the cloned *AAS*$^+$ genes for sequence homology by low stringency cross-hybridization, using DNA fragments known to contain coding sequences of the *AAS* genes as probes (see Fig. 1). The results of low stringency hybridization of these probes to nitrocellulose-bound *Eco*RI digests of the *AAS*$^+$ plasmids and also to wild-type genomic DNA are shown in Fig. 2. The *AAS1* probe hybridizes only with the expected three *Eco*RI fragments of pAH15. Likewise, the *AAS2* probe hybridizes only with the expected single fragments of the three *AAS2*$^+$-containing plasmids pAH16, pAH17, and pAH19. These results alone suggest that the three *AAS* genes do not constitute a set of related DNA sequences. The *AAS3* probe hybridizes not only with the 3.4-kb *Eco*RI fragment of pAH20 that encompasses the *AAS3* probe but also with certain fragments from the other plasmids. In spite of this cross-hybridization (which is probably attributable to a repeated delta sequence present on the *AAS3* probe), there is no detectable homology between the coding sequences of *AAS3* and those of *AAS1* and *AAS2*, because no hybridization occurs between the *AAS3* probe and the cloned sequences carried by pAH15 (*AAS1*) and pAH19 (*AAS2*).

The hybridization of the *AAS1* probe with a single *Bam*HI fragment of the expected size in genomic DNA (Fig. 2, lane G, *AAS1* blot) indicates that *AAS1* is a unique DNA sequence. The *AAS2* and the *AAS3* probes hybridize with multiple genomic fragments (Fig. 2, lane G, *AAS2* and *AAS3* blots). However, at least for *AAS2*, there is a genomic fragment of the expected size exhibiting intense hybridization to the *AAS2* probe.

Mapping of the *AAS* Loci by Using the Cloned *AAS* Genes. We have used the transformant strains described above, which carry integrated copies of pAH15, pAH16, and pAH20, in the 2-μm mapping technique developed by S. C. Falco and D. Botstein (personal communication) to identify the chromosomes on which *AAS1*, *AAS2*, and *AAS3* reside. These genes were then localized by standard tetrad analysis. The mapping data are presented in Fig. 3.

Diploids constructed by crossing an *aas3-1* haploid by a strain bearing *arg9*$^-$, another centromere-linked mutation on chromosome V, have an Aas$^-$ phenotype. Because both *arg9* and *aas3-1* are recessive, their failure to complement indicates that these two mutations are in the same gene. In agreement with this conclusion, no recombinants were observed in 40 tetrads obtained from such a diploid. Moreover, *arg9*$^-$ haploid strains show the phenotypes characteristic of *aas3*$^-$ (see Table 1), and *aas3*$^-$ strains have the weak arginine requirement of *arg9*$^-$

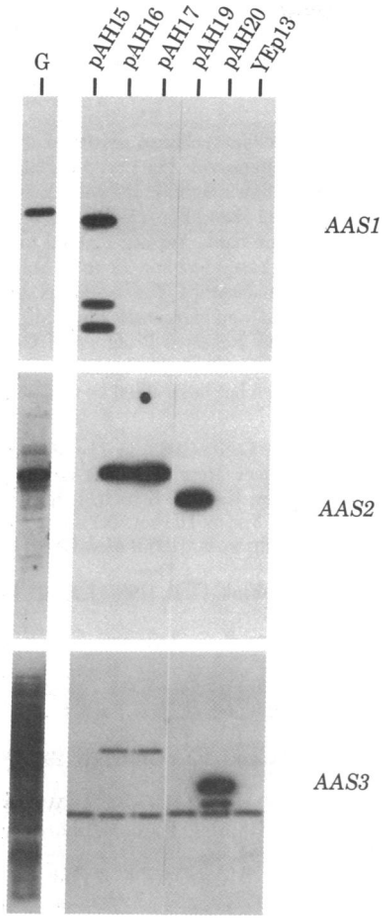

FIG. 2. Southern analysis of genomic DNA and AAS^+ plasmids by using AAS^+ probes. The following digested DNA samples were fractionated by electrophoresis on a 0.6% agarose gel and then blotted to nitrocellulose: genomic DNA (G) digested with *Bam*HI, *Eco*RI, or *Bam*HI and *Bgl* II, in *AAS1*, *AAS2*, and *AAS3* blots, respectively, and plasmids pAH15–pAH17, pAH19, pAH20, and YEp13, all digested with *Eco*RI. The DNA fragments used as probes (Fig. 1) were isolated from low-melting agarose gels and labeled by nick-translation. Hybridization was carried out in 0.6 M NaCl/0.06 M sodium citrate/0.01 M Tris·HCl, pH 7.5/0.1% $NaDodSO_4$/10× concentrated Denhardt's solution at 50°C. Filters were washed in 30 mM NaCl/3 mM sodium citrate/0.1% $NaDodSO_4$ at 30°C.

strains. *ARG9* does not encode a step in the arginine biosynthetic pathway (1). In fact, our results indicate that a mutation in this gene indirectly affects arginine biosynthesis by impairing the general amino acid control over the expression of the arginine pathway.

DISCUSSION

The interactions between the different *aas* mutations and the regulatory mutation *tra3-1* provide insights into the roles of these different gene products in general amino acid control. The *tra3-1* mutation, which leads to constitutive derepression of general control genes, masks the nonderepressible phenotype of mutations in *AAS1*, *AAS2*, and *NDR1*. This epistasis indicates that the positive functions of these *AAS* genes are not required for derepression when the *TRA3* gene product is defective. One interpretation of this result is that these gene products act indirectly to cause derepression by disabling the repressive effect of *TRA3* (9). In contrast to these interactions, *aas3 tra3-1* double mutants exhibit the inability to derepress of *aas3* single mutants. This epistatic relationship shows that *AAS3* function is

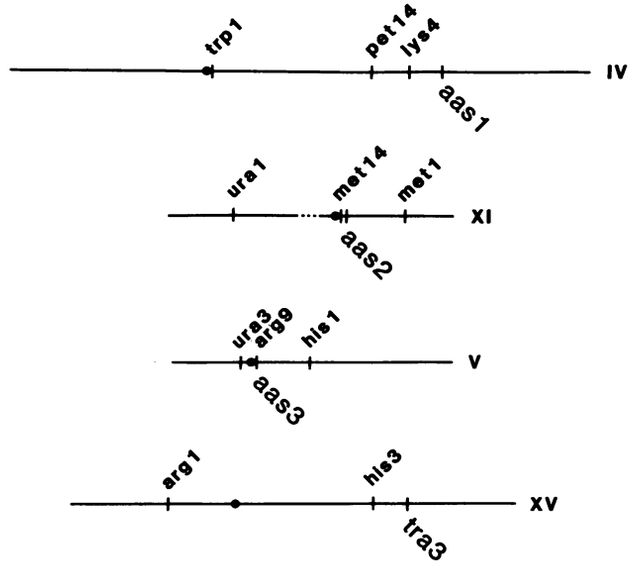

FIG. 3. Genetic mapping of AAS^+ genes. Partial genetic maps of chromosomes IV, XI, and V are shown, based on the following tetrad data (expressed as PD:NPD:TT): *aas1-lys4*, 55:2:42; *aas1-pet14*, 20:1:49; *aas2-met14*, 95:0:10; *aas3-ura3*, 45:0:9. The *pet14-lys4-aas1* gene order was established by a consideration of the minimal number of crossovers needed to generate the observed recombinants in a three-point cross. The 2-μm mapping argues strongly that *aas2* is on the right arm of XI (see text). A partial map of XV is also shown, giving the location of the *TRA3* gene (15).

needed for derepression even when *TRA3* is inactive and that the *AAS3* product acts more directly to affect derepression than the products of other *AAS* genes.

The $AAS3^+$ gene, when placed on an episomal plasmid, complements mutations in *AAS1* and *AAS2*, nearly as well as *aas3* mutations. Our interpretation of this result is that the *AAS1* and *AAS2* products are positive regulators of *AAS3* and that the end result of their function is to increase the level of active *AAS3* product in the cell. Assuming that *AAS1* and *AAS2* are negative regulators of *TRA3*, we suggest that *TRA3* also acts indirectly and exerts its repressive effect on gene expression as a negative regulator of *AAS3*. These proposed regulatory interactions are summarized in Fig. 4. According to this scheme, increasing *AAS3* dosage titrates out the *TRA3* product, leading to a net level of *AAS3* activity that normally occurs only when *AAS1* and *AAS2* function collectively to inhibit the *TRA3* product during amino acid starvation. The other *AAS* genes fail to complement an $aas3^-$

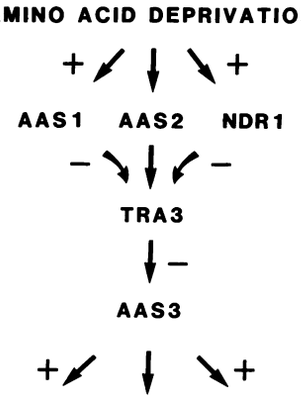

FIG. 4. Regulatory circuit of general amino acid control. Activating effects are depicted as + and inactivation is depicted as −.

strain because the *AAS3* product acts more directly than do the products of these other genes. Although the scheme in Fig. 4 explains all of the available data, it is important to note that other positive regulatory factors might exist, which act to bring about derepression even more directly than the *AAS3* product. Moreover, the *aas-tra3* mutant interactions have been examined with a single *TRA3* allele. Having recently cloned the *TRA3* gene (unpublished data), we will be able to produce new alleles of this locus and test the generality of our model.

There are striking similarities between the regulatory circuit diagramed in Fig. 4 and those that have been proposed for the regulation of the galactose utilization genes of *S. cerevisiae*, and the phosphatase genes of both *S. cerevisiae* and *Neurospora crassa* (16). Genetic analysis suggests that in each of these systems, the proximal effectors are positive regulators of gene expression and that the negative regulatory factors exert their effects indirectly. In addition, in the phosphatase systems, as in general amino acid control, additional positive factors have been identified that also function indirectly by antagonism of the negative effector(s).

For the galactose regulatory system, there is good evidence that the negative and positive factors (the *GAL80* and *GAL4* products, respectively) interact with one another to determine the level of activity of the positive factor [the *GAL4* product (16–18)]. In the case of general amino acid control, the fact that we have isolated *aas2* and *aas3* mutations as functional suppressors of the temperature sensitivity associated with the *tra3-1* mutation argues in favor of a physical interaction between the *TRA3* product and the *AAS2* and *AAS3* gene products. In this view, a destabilizing alteration in the *TRA3* product is corrected by a compensatory mutation in an *AAS* molecule with which it normally interacts. The lethality of the *aas2-1 tra3-1* double mutant can also be understood as a physical interaction between the gene products, at least one of which is essential. If this explanation is correct, it would indicate that the interactions between regulatory factors shown in Fig. 4 occur at the level of the regulatory gene products themselves and not by regulation of gene expression.

We have recently found that transformants containing $AAS3^+$ on a high copy episome are partially derepressed for *HIS4* expression in the absence of amino acid starvation (unpublished data). Our finding that *aas3* mutants are leaky arginine auxotrophs further suggests that $AAS3^+$ is required to maintain the basal level of expression of *ARG* genes. The fact that expression can be modulated simply by altering *AAS3* gene dosage is consistent with a direct interaction between the *AAS3* product and the structural genes it regulates. The sites of positive control recently identified in the *HIS3* and *HIS4* promoters (4, 5) are likely recognition sites for the *AAS3* product. The availability of the $AAS3^+$ gene makes it possible to test this aspect of our model.

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