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Genetic Evidence for Functional Interactions Between Actin Noncomplementing (Anc) Gene Products and Actin Cytoskeletal Proteins in *Saccharomyces cerevisiae*

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ABSTRACT

We describe here genetic interactions between mutant alleles of Actin-NonComplementing (*ANC*) genes and actin (*ACT1*) or actin-binding protein (*SAC6, ABP1, TPM1*) genes. The *anc* mutations were found to exhibit allele-specific noncomplementing interactions with different *act1* mutations. In addition, mutant alleles of four *ANC* genes (*ANC1, ANC2, ANC3* and *ANC4*) were tested for interactions with null alleles of actin-binding protein genes. An *anc1* mutant allele failed to complement null alleles of the *SAC6* and *TPM1* genes that encode yeast fimbrin and tropomyosin, respectively. Also, synthetic lethality between *anc3* and *sac6* mutations, and between *anc4* and *tpm1* mutations was observed. Taken together, the above results strongly suggest that the *ANC* gene products contribute to diverse aspects of actin function. Finally, we report the results of tests of two models previously proposed to explain extragenic noncomplementation.

EXTRAGENIC noncomplementation can occur between mutations in genes that encode physically interacting proteins. For example, in both Drosophila and yeast, mutations in α-tubulin genes can fail to complement mutations in β-tubulin genes (HAYS *et al.* 1989; STEARNS and BOTSTEIN 1988). In addition, mutations in yeast genes that encode the actin-binding proteins Sac6p (fimbrin) and Abp1p can fail to complement mutations in the actin gene (WELCH *et al.* 1993). Based on these observations, the *ANC* genes identified in screens for extragenic mutations that fail to complement mutations in the *ACT1* gene (WELCH *et al.* 1993) might encode actin-binding proteins or proteins important for actin function.

To gain information about the roles of the Anc proteins *in vivo*, we sought genetic evidence for interactions between *ANC* gene products and other components of the actin cytoskeleton, and among the *ANC* gene products themselves. The *SAC6, ABP1* and *TPM1* genes which encode the actin-binding proteins fimbrin, Abp1p and tropomyosin, respectively, were chosen for these studies because the proteins that they encode have diverse properties and distinct localizations *in vivo*. Tropomyosin associates with cytoplasmic actin cables (LIU and BRETSCHER 1989b), Abp1p associates with cortical actin structures, and fimbrin associates with both cytoplasmic and cortical structures (DRUBIN, MILLER and BOTSTEIN 1988). Furthermore, fimbrin and tropomyosin have different biochemical activities (ADAMS, BOTSTEIN and DRUBIN 1991; LIU and BRETSCHER 1989a) (the biochemical activities of Abp1p have not been determined). All three genes are nonessential.

Evidence for interactions was sought by identifying examples of extragenic noncomplementation and synthetic phenotypes. Synthetic phenotypes refer here to negative synergistic interactions between two mutations in haploid strains. These interactions often occur between mutations in genes that encode proteins that cooperate in a cellular process (KAISER and SCHEKMAN 1990; SALMINEN and NOVICK 1987; STEARNS, HOYT and BOTSTEIN 1990). Thus, the identification of synthetic phenotypes can be diagnostic of functional relationships between gene products. We show here that the *anc* mutations exhibit multiple genetic interactions with mutant alleles of different actin-binding protein genes, and with a variety of *act1* alleles. These results provide strong evidence that the *ANC* gene products contribute to actin cytoskeletal functions, and indicate that each Anc protein participates in distinct aspects of actin function.

Finally, to increase our understanding of the nature of the interactions between the products of the *ANC* genes and the *ACT1* gene, we tested for allele specificity of noncomplementation using different *act1* and *anc* alleles including, where possible, null alleles. These results bear on the molecular basis for extragenic noncomplementation.

MATERIALS AND METHODS

Strains and media: The yeast strains used in this paper are derivatives of strain S288C and are listed on Table 1.

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TABLE 1

Yeast strains and plasmids

Strain and plasmid	Genotype	Source
Strain		
DDY9	*MAT***a** *tub2-201 ura3 his4*	This laboratory
DDY16	*MAT***a** *act1-1 ura3 his4*	This laboratory
DDY17	*MATα act1-1 ura3 his4*	This laboratory
DDY177	*MAT***a** *act1-2 his4*	SHORTLE, NOVICK and BOTSTEIN (1984)
DDY178	*MAT***a** *act1-4 his4 leu2*	This laboratory
DDY183	*MATα ura3 his4*	Botstein laboratory
DDY184	*MATα leu2*	Botstein laboratory
DDY185	*MAT***a** *leu2*	Botstein laboratory
DDY196	*MATα tpm1::LEU2 leu2 ura3 his3 ade2*	LIU and BRETSCHER (1992)
DDY197	*MAT***a** *tpm1::URA3 ura3 his4*	LIU and BRETSCHER (1989b)
DDY198	*MATα tpm1::URA3 ura3 lys2*	LIU and BRETSCHER (1989b)
DDY207	*MATα act1-4 ura3 ade2*	DUNN and SHORTLE (1990)
DDY208	*MATα act1-1 lys2*	SHORTLE, NOVICK and BOTSTEIN (1984)
DDY216	*MATα sac6::URA3 ura3 lys2 his3 leu2 trp1*	ADAMS, BOTSTEIN and DRUBIN (1989)
DDY217	*MAT***a** *sac6::URA3 ura3 lys2 his3 leu2 trp1*	ADAMS, BOTSTEIN and DRUBIN (1989)
DDY262	*MAT***a** *abp1::LEU2 leu2 ura3 lys2 ade2*	This laboratory
DDY267	*MAT***a** *act1-4 ura3 ade2*	This study[a]
DDY268	*MATα ura3 ade2*	This study[a]
DDY269	*MATα act1-4 ura3 ade2*	This study[a]
DDY273	*MATα act1-4 his4 leu2*	This study[b]
DDY299	*MATα anc2-1 ura3 leu2*	This study
DDY300	*MATα anc4-1 ura3 his4*	This study
DDY349	*MATα act1-124::HIS3 his3 ura3 leu2 tub2-201*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY350	*MATα act1-125::HIS3 his3 ura3 leu2 tub2-201 can1*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY351	*MATα act1-129::HIS3 his3 ura3 leu2 tub2-201 can1*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY362	*MAT***a** *anc1Δ1::HIS3 his3 ura3 leu2 can1*	This study
DDY363	*MATα anc1Δ1::HIS3 his3 ura3 leu2 can1 cry1*	This study
DDY365	*MAT***a** *anc1-2 ura3 leu2*	This study
DDY375	*MAT***a** *act1-1 lys2*	This laboratory
DDY377	*MAT***a** *his3 leu2 ura3*	This laboratory
DDY378	*MAT***a** *ura3 lys2*	This laboratory
DDY379	*MATα ura3 lys2*	This laboratory
DDY382	*MAT***a** *ANC1:URA3:ANC1 ura3 his4*	This study
DDY384	*MATα act1Δ1::LEU2 leu2 ura3 tub2-201 can1 his3* (pKFW29)	This laboratory[c]
DDY385	*MAT***a** *act1Δ1::LEU2 leu2 ura3 tub2-201 his3 ade2* (pKFW29)	This laboratory[c]
DDY386	*MATα act1-108::HIS3 his3 ura3 leu2 tub2-201*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY387	*MATα act1-111::HIS3 his3 ura3 leu2 tub2-201 ade2*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY388	*MATα act1-112::HIS3 his3 ura3 leu2 tub2-201 ade4*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY389	*MATα act1-122::HIS3 his3 ura3 leu2 tub2-201 ade4*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY390	*MATα ACT1::HIS3 his3 ura3 leu2 ade4*	WERTMAN, DRUBIN and BOTSTEIN (1992)
DDY391	*MAT***a** *ura3 his4 lys2*	This laboratory
DDY392	*MAT***a** *his3 leu2 ura3*	This laboratory
DDY393	*MAT***a** *hmg1::LYS2 lys2 ura3 his3 ade2 met1*	BASSON *et al.* (1987)
DDY394	*MATα hmg2::HIS3 his3 ura3 lys2 ade2*	BASSON *et al.* (1987)
DDY395	*MAT***a** *anc1-1 ura3 his4*	This study
DDY396	*MATα anc1-1 ura3 his4*	This study
DDY397	*MATα anc1-2 ura3*	This study
DDY398	*MATα anc1-2 ura3 his4*	This study
DDY399	*MAT***a** *anc1-2 ura3 his4*	This study
DDY400	*MATα anc2-1 ura3 his4 lys2*	This study
DDY401	*MAT***a** *anc2-1 ura3 leu2 lys2*	This study
DDY402	*MAT***a** *anc2-1 ura3 his4*	This study
DDY403	*MATα anc3-1 ura3 leu2*	This study
DDY404	*MATα anc3-1 ura3 his4 leu2*	This study
DDY405	*MATα anc3-1 ura3 his4*	This study
DDY406	*MAT***a** *anc3-1 ura3 his4*	This study
DDY407	*MATα anc4-1 ura3 leu2 lys2*	This study
DDY408	*MAT***a** *anc4-1 ura3 leu2 lys2*	This study
DDY409	*MATα anc4-1 ura3 his4*	This study

TABLE 1—Continued

Yeast strains and plasmids

Strain and plasmid	Genotype	Source
DDY410	*MATα nc50 leu2*	This study
DDY411	*MATα nc50 ura3 leu2*	This study
DDY412	*MATα nc51 leu2*	This study
DDY413	*MAT***a** *nc51 ura3 leu2*	This study
DDY414	*MATα nc52 ura3 his4 leu2*	This study
DDY415	*MATα nc53 ura3 leu2*	This study
DDY416	*MAT***a** *nc54 ura3 his4 lys2*	This study
DDY417	*MAT***a** *nc55 ura3 his4*	This study
DDY418	*MAT***a** *nc56 ura3 his4 leu2*	This study
DDY419	*MAT***a** *nc57 ura3 his4*	This study
DDY486	*MAT***a** *nc58 ura3 leu2*	This study
DDY487	*MATα ANC4 tpm1::LEU2 leu2 ura3 lys2* (pDD20)	This study[d]
DDY488	*MAT***a** *anc4-1 TPM1 leu2 ura3 lys2* (pDD20)	This study[d]
DDY489	*MATα ANC4 TPM1 leu2 ura3 ade2* (pDD20)	This study[d]
DDY490	*MAT***a** *anc4-1 tpm1::LEU2 leu2 ura3 ade2* (pDD20)	This study[d]
DDY491	*MATα act1-1 ANC1:URA3:ANC1 ura3 his4*	This study[e]
DDY492	*MATα act1-4 ANC1:URA3:ANC1 ura3 ade2*	This study[f]
DDY493	*MAT***a**/α *act1-4/act1-4 ura3/ura3 his3/his3 lys2/LYS2 leu2/LEU2 ADE2/ade2*	This laboratory
Plasmid		
pDD20	*TPM1* on YEp52, *GAL* promoter	LIU and BRETSCHER (1989b)
pKFW29	*ACT1* on YCp50	This laboratory

[a] Segregant from backcrossing DDY207 to our laboratory strain (DDY185) three times.
[b] Segregant from DDY178 × DDY184.
[c] Segregant from KWY201 (WERTMAN, DRUBIN and BOTSTEIN 1992) transformed with pKFW29.
[d] Tetrad segregant from DDY196 crossed to DDY408 (pDD20).
[e] Segregant from DDY382 crossed to DDY17.
[f] Segregant from DDY382 crossed to DDY269.

Media for yeast growth and sporulation were as described by ROSE, WINSTON and HIETER (1990). YPD is yeast complex medium and SD is synthetic medium that was supplemented with appropriate nutrients. Preparation of 5-fluoroorotic acid (5-FOA) plates was as described by BOEKE, LACROUTE and FINK (1984).

Genetic techniques and transformation: Yeast mating, sporulation and tetrad analysis were performed as described by ROSE, WINSTON and HIETER (1990). Growth was assayed by spotting suspensions of cells in water onto agar plates. Yeast cells were transformed with DNA by the lithium acetate method (ITO *et al.* 1983) as modified by SCHIESTL and GIETZ (1989). Transformants were plated on SD medium lacking uracil to select cells that acquired the plasmid.

Plasmid construction: Haploid strains DDY384 and DDY385 (*act1Δ1::LEU2*) were derived from *act1Δ1::LEU2/ACT1* diploids, containing pKFW29, that were constructed as described in WERTMAN, DRUBIN and BOTSTEIN (1992). pKFW29 is a derivative of YCp50 (ROSE *et al.* 1987) which contains at the *Eco*RI site, a 3.8-kb yeast genomic *Eco*RI fragment bearing the *ACT1* gene (NG and ABELSON 1980). The inserted fragment is oriented such that *ACT1* transcription proceeds in the same direction as β-lactamase.

Complementation test of the *act1* null allele: Haploid cells carrying a deletion of the *ACT1* gene [*act1Δ1::LEU2*] are inviable unless they also carry a CEN, *URA3*-plasmid that contains the *ACT1* gene (in this case, pKFW29). To test for complementation of the *act1* null allele, *anc* mutants (Leu^-) were crossed to *act1Δ1::LEU2* strains containing the Ura^+ plasmid (DDY384 or DDY385) and diploids were selected on SD medium lacking uracil and leucine. Diploid cells (*anc/ANC act1Δ1::LEU2/ACT1*) were transferred onto 5-FOA plates at 25° to select for cells that had lost the Ura^+ plasmid. The loss of the plasmid was confirmed by streaking cells on SD plates lacking uracil. Cells were then restreaked and allowed to grow into single colonies on YPD plates before scoring for complementation at the restrictive temperature (37°).

Identification of double mutant strains: Diploid strains doubly heterozygous for mutations in two *ANC* genes, or in an *ANC* gene and an actin-binding protein gene, were generated by crossing appropriate haploids. These diploids were sporulated and the resulting tetrads were dissected to isolate double mutants. Segregation of *anc* mutations was followed by their temperature-conditional growth: *anc2* and *anc4* fail to grow at 37°, and *anc3* fails to grow at 14°. The segregation of other mutations was evaluated by following the nutritional markers that are linked to the gene disruptions: *anc1Δ1::HIS3*, *abp1::LEU2*, *tpm1::URA3* or *tpm1::LEU2*, and *sac6::URA3*. In the cases where double mutants of two Ts^- mutations were constructed, the Ts^- spore colonies from NPD tetrads were deduced to be the double mutants. For some crosses, *anc* mutations were also identified by their failure to complement known *anc* mutations in tester strains. To perform these tests, cells from spore colonies were spotted on lawns of *anc* mutants of both mating types, allowed to mate overnight at 20°, and then transferred to the nonpermissive temperatures. Growth at 37° was scored after 36–48 hr, and growth at 14° was scored after 3–5 days.

Determination of the *anc tpm1* double mutant phenotype: To construct *anc tpm1* double mutants, it was necessary to transform *anc/ANC tpm1::LEU2/TPM1* cells with a plasmid carrying the *TPM1* gene to suppress the high incidence of spore inviability that is characteristic of *ANC tpm1* and *anc tpm1* mutant spores. *anc* mutants were crossed to the

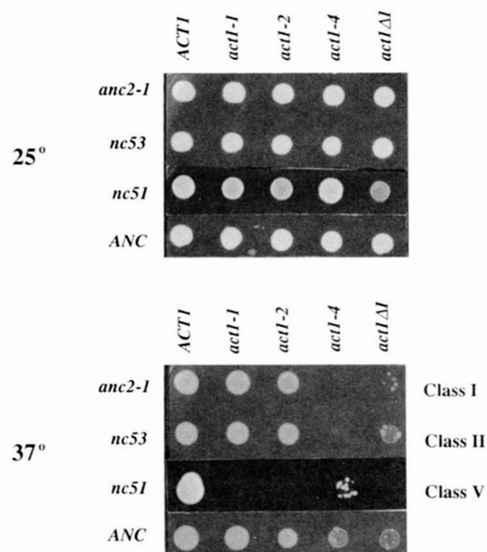


FIGURE 1.—Allele specificity of *act1* noncomplementation by *anc* mutations. *anc/ANC act1/ACT1* diploids were assayed for growth at 25° and 37°. Cells were spotted on YPD plates using a multipoint inoculator. Photographs were taken after 2–3 days for growth at 25° and after 36–48 hr for growth at 37°. Classes of allele specificity are as described in Table 2.

tpm1::LEU2 strain (DDY196) and the diploids were transformed with a 2μ, *URA3*-plasmid that bears the wild-type *TPM1* gene (pDD20). Doubly heterozygous cells were sporulated, their tetrads were dissected and grown at 25°. The viability of these spore colonies was similar when tetrads were dissected on nonselective (YPD) or selective (SD lacking uracil) medium. To assess the growth of *anc tpm1* double mutants, spore colonies were spotted on 5-FOA plates at 25° to select for cells that had lost the Ura$^+$ plasmids. Growth on 5-FOA plates was scored after 3–5 days.

RESULTS

Allele specificity of *act1* noncomplementation: To determine whether *anc-act1* noncomplementation is allele specific, *anc/ANC act1/ACT1* double heterozygotes were created by crossing *anc* mutants to the *act1-1* (DDY208), *act1-2* (DDY177), *act1-4* (DDY267) or *act1Δ1::LEU2* (DDY384) strains. For these studies, two different strains carrying each *anc* mutation were crossed to at least two different strains containing the same *act1* allele. *act1-1* and *act1-2* are recessive temperature-sensitive alleles (SHORTLE, NOVICK and BOTSTEIN 1984), *act1-4* is a semidominant temperature-sensitive allele (DUNN and SHORTLE 1990) (see below), and *act1Δ1::LEU2* is a null allele. The temperature-conditional phenotypes of the *anc* mutations used in these studies are recessive [(WELCH *et al.* 1993) and see Figure 1 and Table 3]. Complementation tests for all diploids were performed by spotting cells on YPD plates and assaying for growth at the permissive (25°) and nonpermissive temperatures (37°). Mutations in *ANC* and *ACT1* genes were considered noncomplementing if the double heterozygote (*anc/ANC act1/ACT1*) was found to grow less well than the single heterozygote (*ANC/ANC act1/ACT1*). As shown in Figure 1, different *act1* heterozygotes have different growth rates at 37°; *act1–1/ACT1, act1–2/ACT1* and *ACT1/ACT1* growth rates are indistinguishable, while *act1–4/ACT1* and *act1Δ1::LEU2/ACT1* cells grow more slowly.

TABLE 2

Allele specificity of *act1* noncomplementation

anc mutation[a]	Growth of heterozygotes[b]				Complementation class[c]
	act1-1	*act1-2*	*act1-4*	*act1Δ1::LEU2*	
ANC	+	+	±	±(p)	NA
anc2-1	+	+	−	−(p)	I
nc52	+	+	−	−	I
nc54	+	+	−	−	I
nc56	+	+	−	−	I
nc53	+	+	−	±(p)	II
nc57	+	+	−	±(p)	II
nc58	+	+	−(p)	±(p)	II
anc3-1	+	±(p)	−	−	III
anc4-1	+	±	−(p)	−(p)	III
nc55	+	±	−	−(p)	III
anc1-1	−(p)	−(p)	−	−	IV
anc1-2	−(p)	−	−	−	IV
anc1Δ1::HIS3	−(p)	−(p)	−	−	IV
nc50	−	−	−(p)	−	V
nc51	−	−	−(p)	−	V

[a] *anc1-1, anc1-2, nc50,* and *nc51* were isolated in the screen with *act1-1*, and the remaining mutants were isolated in the screen with *act1-4* (WELCH *et al.* 1993). *ancX* are mutations that exhibit a recessive temperature-conditional phenotype (see text), while *ncX* are mutations that do not. *ancX* mutations were assigned to linkage groups while *nc* mutations were not.

[b] *anc/ANC act1/ACT1* diploids were generated and tested for growth at 37°. Growth rates are indicated as follows: +, thick uniform spot; ±, thin uniform spot; −, no growth. (p) means papillation.

[c] *anc* mutations were grouped into classes based on their ability to complement different *act1* alleles. For each *act1* allele tested, the growth rate of double heterozygotes (*anc/ANC act1/ACT1*) was standardized against the growth rate of *act1/ACT1* and *anc/ANC* single heterozygotes. Class I mutations complement *act1-1* and *act1-2*, but not *act1-4* or *act1Δ1::LEU2*; class II mutations complement *act1-1, act1-2* and *act1Δ1::LEU2*, but not *act1-4*; class III mutations complement *act1-1*, but not *act1-2, act1-4,* or *act1Δ1::LEU2*; class IV mutations fail to complement *act1-1* and *act1-2* less severely than *act1-4* or *act1Δ1::LEU2*; class V mutations fail to complement *act1-4* less severely than *act1-1, act1-2,* or *act1Δ1::LEU2*. NA, not applicable.

Fourteen *anc* mutations were characterized (Table 2). [The term "*anc*" is used here and elsewhere as a generic term to refer to all extragenic actin noncomplementing mutations. In cases where a specific allele number is designated, "*anc*" refers to mutations that confer temperature-conditional lethality, and "*nc*" refers to mutations that do not. In the context where allele numbers are designated, *anc* mutations, but not *nc* mutations, have been assigned to linkage groups (WELCH *et al.* 1993).] The *anc* mutations can be placed into five classes based on the results of complementation tests with different *act1* mutations. Classes I, II and III are mutations isolated in the noncomplemen-

TABLE 3

Complementation behavior of *anc* mutations with alanine-series *act1* mutants

act1 allele[a]	Residue change[b]	*anc* mutation					
		ANC	*anc1-1*	*anc1-2*	*anc2-1*	*anc3-1*	*anc4-1*
ACT1::HIS3	NA	+	+	+	+	+	+
act1-108	R256A, E259A	±	−	−	−	−	−
act1-111	D222A, E224A, E226A	±(p)	−	−	±	−	−
act1-112	K213A, E214A, K215A	±(p)	−	−	±	−	−
act1-122	D80A, D81A	+	±	±(p)	+	±(p)	+
act1-124	D56A, E57A	+	+	+	+	+	+
act1-125	K50A, D51A	+	+	+	+	+	+

Complementation was determined by analysing the growth of double heterozygotes at 37° on YPD after 36–60 hr. The assignment of + or − for growth rate is similar to that described in Table 2.

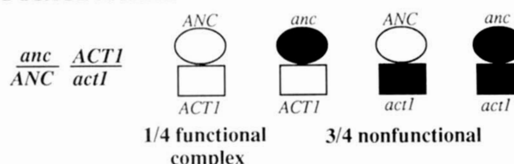
[a] The *act1* alleles are designated as in WERTMAN, DRUBIN and BOTSTEIN (1992).

[b] Residues changed in each *act1* mutation are indicated. For example, [R256A, E259A] means that both the arginine at position 256 and the glutamate at position 259 are changed to alanine. *act1-108, 111* and *112* alleles change residues proximal to residue changed in *act1-4* (E259V) in the tertiary structure of actin; *act1-122, 124* and *125* change residues proximal to residue changed in *act1-1* (P32L). NA, not applicable.

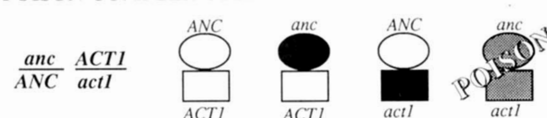
tation screen against *act1-4* and differ in their ability to complement *act1-2* and *act1Δ1::LEU2*. Class I and III mutations failed to complement *act1Δ1::LEU2*, but class III mutations also failed to complement *act1-2*. In contrast, class II mutations could complement both *act1-2* and *act1Δ1::LEU2*. Finally, classes IV and V consist of *anc* mutations isolated in the *act1-1* screen. These mutations failed to complement all *act1* alleles, but the severity of noncomplementation varied with the *act1* alleles. Examples of class I, II and V mutants are shown in Figure 1.

The five recessive conditional-lethal *anc* mutations (*anc1-1, anc1-2, anc2-1, anc3-1* and *anc4-1*) were also tested for their ability to complement six recessive temperature-sensitive *act1* alleles that were created by replacing charged residues with alanine (WERTMAN, DRUBIN and BOTSTEIN 1992). These alleles were chosen because they change residues that, in the tertiary structure of actin (KABSCH *et al.* 1990), are located near residues changed by the *act1-1* (P32L) (SHORTLE, NOVICK and BOTSTEIN 1984) and *act1-4* (E259V) (DUNN and SHORTLE 1990) mutations (Table 3). *act1-108, act1-111* and *act1-112*, which change residues in proximity to the residue changed in *act1-4*, caused semidominant temperature-sensitivity similar to that observed for *act1-4* (Figure 1). [WERTMAN, DRUBIN and BOTSTEIN (1992) reported that the *act1-111* and *act1-112* alleles cause a recessive Ts⁻ phenotype. The discrepancy between our findings and those reported by WERTMAN *et al.* might be due to differences in the genetic backgrounds of the wild-type strains used in each study or to minor variations in the incubation temperature.] In contrast, *act1-122, act1-124* and *act1-125* alleles, which change residues close to the residue changed in *act1-1*, are completely recessive for their temperature-sensitive phenotypes, similar to *act1-1*. Table 3 shows that all of the *anc* mutations (except

A. DOSAGE MODEL



POISON-COMPLEX MODEL



B. Test for Poison-complex model

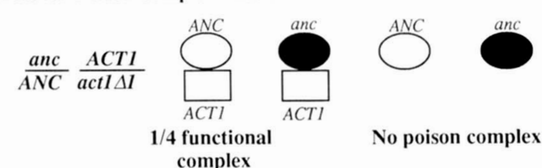


FIGURE 2.—(A) The Dosage and Poison-complex models that explain extragenic noncomplementation (adapted after STEARNS and BOTSTEIN 1988). For simplicity, only binary interactions between *ANC* and *ACT1* gene products are illustrated. See DISCUSSION for explanation. (B) The Poison-complex model predicts that *anc/ANC act1Δ1/ACT1* cells grow because a poison complex does not form. Oval box, Anc protein; rectangle box, actin protein; white fill = wild-type; black or gray fill = mutant.

anc2-1) failed to complement *act1-108, act1-111* and *act1-112*. In addition, *anc1-1, anc1-2* and *anc3-1* displayed a weak noncomplementation phenotype with *act1-122*. All other combinations of *anc-act1* mutations complemented each other.

Tests of genetic models for *act1* extragenic noncomplementation: Two models proposed to explain extragenic noncomplementation are diagrammed in Figure 2A (FULLER *et al.* 1989; STEARNS and BOTSTEIN 1988) and will be considered in more detail in the DISCUSSION. The poison-complex model predicts

that the extragenic noncomplementing phenotype requires the presence of both mutant gene products. To determine whether noncomplementation requires the expression of a mutant actin protein (Figure 2B), we generated *anc/ANC act1Δ1::LEU2/ACT1* diploid cells and tested their growth at 37°. As indicated in Table 2, most of the *anc* and *nc* mutations fail to complement the *act1Δ1::LEU2* null allele, indicating that in these cases the noncomplementation phenotype does not require the presence of mutant actin. For one of the *anc* mutants (*anc1*) which fails to complement the *act1Δ1::LEU2* allele, we also determined whether noncomplemention requires the presence of mutant Anc1p. We tested whether *anc1Δ1::HIS3* (DDY362; M. Welch and D. Drubin, unpublished results), a deletion of *ANC1* that causes a slow growth phenotype at 25° and failure to grow at 37°, could complement *act1-1*, *act1-2*, *act1-4* and *act1Δ1::LEU2* (Table 2 and Figure 3). In all cases, the *anc1Δ1-act1* double heterozygotes were inviable at 37°, demonstrating that the noncomplementation phenotype does not require the presence of mutant Anc1p.

To further characterize the genetic interaction between the *anc1* mutation and *act1* alleles, we tested whether increasing the dosage of the *ANC1* gene product would suppress the noncomplementation phenotype of *anc1-2/ANC1 act1/ACT1* strains. Strains which contain a chromosomal duplication of *ANC1* (DDY382; M. Welch and D. Drubin, unpublished results) were used. Duplication of *ANC1* did not affect the growth of *ACT1/ACT1* cells. As shown in Figure 3, an additional copy of *ANC1* (*anc1-2/ANC1:URA3:ANC1 act1-1/ACT1* strain) suppressed the noncomplementation phenotype. Interestingly, an additional copy of *ANC1* was unable to suppress the noncomplementation phenotype of *anc1-2/ANC1 act1-4/ACT1* diploids, and *anc1Δ1::HIS3/ANC1:URA3:ANC1 act1-4/ACT1* cells did not grow as well as *act1-4/ACT1* cells at 37°. These observations suggest that the *ANC1* duplication results in elevated but not wild-type levels of expression of Anc1p.

In contrast to the results for *anc1*, the *nc53*, *nc57* and *nc58* mutations could complement *act1Δ1::LEU2* (Table 2, Figure 1). In each case, the growth rate of the double heterozygote was indistinguishable from the growth rate of the *act1Δ1::LEU2/ACT1* single heterozygote. These results show that the noncomplementation phenotype requires the presence of a mutant actin, consistent with the possibility that noncomplementation results from poison complex formation. A prediction made by Stearns and Botstein (1988) is that elevation of wild-type subunit levels might not suppress the mutant phenotype caused by a poison protein complex. Therefore, to provide additional information about the basis of the noncomplementation phenotype observed for the *nc53*, *nc57* and *nc58* mutations, we determined whether an additional copy of the *ACT1* gene could suppress the phenotype of double heterozygotes generated from crossing *nc53*, *nc57* and *nc58* strains to an *act1-4* strain. Each diploid strain was transformed with a CEN-*ACT1* plasmid (pKFW29) or with a control plasmid lacking the *ACT1* gene (pRS316; Sikorski and Hieter 1989). In each case, pKFW29, but not the control plasmid, suppressed the noncomplementation phenotype (Figure 4).

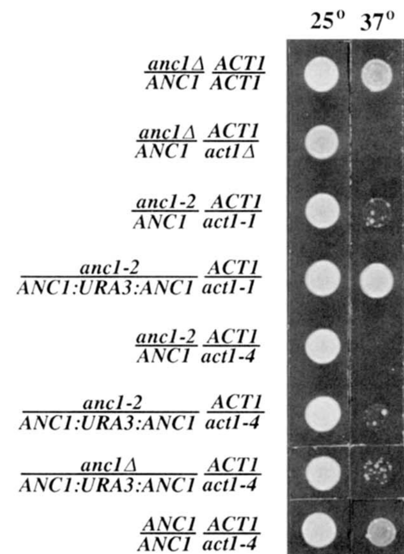


Figure 3.—Evidence for the Dosage model. An additional copy of *ANC1*, but not the deletion of *ANC1*, is able to suppress the noncomplementing phenotype between *anc1-2* and *act1-1*. Various combinations of *anc1-2/act1-1* and *anc1-2/act1-4* double heterozygotes were generated. Cells were grown on YPD plates and scored after 40 hr at both 25° and 37°.

Additional examples of extragenic noncomplementation: We sought to identify potential interactions between *ANC* gene products and actin-binding proteins by testing for extragenic noncomplementation. We tested the ability of the five conditional-lethal *anc* mutations to complement *abp1::LEU2* (DDY262), *sac6::URA3* (DDY217) and *tpm1::LEU2* (DDY196). These are null alleles of nonessential genes that encode actin-binding proteins (Holtzman, Yang and Drubin 1993; Adams, Botstein and Drubin 1989; Liu and Bretscher 1989b). *sac6::URA3* and *tpm1::LEU2* mutations cause recessive temperature sensitivity at 37° and *abp1::LEU2* does not cause a detectable mutant phenotype. We mated the *anc* mutants to *abp1*, *sac6* and *tpm1* null strains to generate different combinations of double heterozygotes, and analyzed the growth of each strain at 37° and 14°. The growth of these double heterozygotes at the restrictive temperatures was standardized against the growth of *anc/ANC ABP/ABP* and *ANC/ANC abp/ABP* single heterozygotes (here, *ABP* denotes all actin-binding protein genes mentioned above). With the excep-

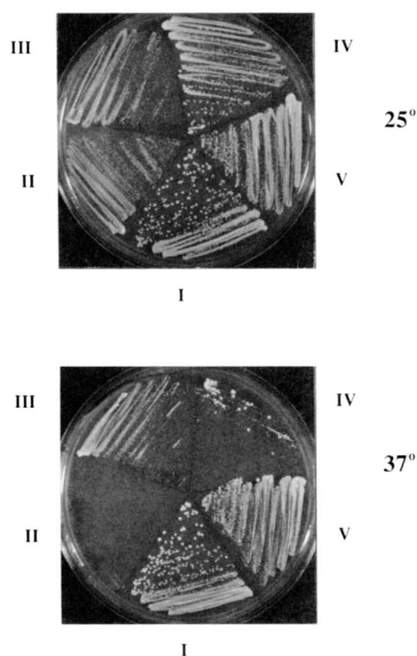


FIGURE 4.—A plasmid-borne copy of *ACT1* suppresses the noncomplementation phenotype. Diploid cells (see below) were transformed with a CEN-plasmid containing either the *ACT1* gene (pKFW29) or no insert (pRS316). Stably transformed colonies were streaked on SD plates and tested for growth at 25° (top panel) and 37° (bottom panel). Photographs were taken 3 days after streaking. Cells from the same numbered sector in both panels were derived from the same colony. I, wild-type [DDY180 × DDY379]; II, *act1-4/act1-4* [DDY393] (pRS316); III, *act1-4/act1-4* (pKFW29); IV, *nc57/NC57 act1-4/ACT1* [DDY419 × DDY469] (pRS316); V, *nc57/NC57 act1-4/ACT1* (pKFW29).

tion of mutations in the *ANC1* gene, all combinations of *anc* and *abp* mutations complemented each other at 14° and 37°. Both *anc1–2* and the null allele *anc1Δ1::HIS3* could complement *abp1::LEU2*. However, *anc1–2* only weakly complemented *sac6::URA3* and *tpm1::LEU2*, while *anc1Δ1::HIS3* failed to complement either mutation (Table 4). *anc* mutants were also tested for their ability to complement each other at the restrictive temperatures. All pairwise combinations of *anc* mutations complemented each other, except for *anc1-2* and *anc3-1* mutations which complemented each other weakly at 14°, and did not complement each other at 37° (Table 4).

As controls for the above experiments, we crossed the *anc* mutants to strains containing mutations (*tub2-201, hmg1::LYS2* or *hmg2::HIS3*) in genes that do not participate in actin functions, and determined whether the double heterozygotes grow at 14° and 37°. *TUB2* encodes β-tubulin and *HMG1* and *HMG2* encode different isoforms of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (BASSON *et al.* 1987). All control diploids grew as well as wild-type strains at both 14° and 37° (data not shown).

Synthetic phenotypes of double mutants: The phenotypes of haploid *anc-anc* or *anc-abp* double mutants were determined. To create double mutant strains, we dissected tetrads generated from dipoid strains

TABLE 4

Noncomplementation between *anc* mutations and between *anc* and actin-binding protein mutations

Heterozygote[a]	Growth at 14°	25°	37°
anc1-2/ANC1 anc3-1/ANC3	±	+	−
anc1-2/ANC1 sac6/SAC6	+	+	±
anc1Δ1/ANC1 sac6/SAC6	+	+	−
anc1-2/ANC1 tpm1/TPM1	+	+	±
anc1Δ1/ANC1 tpm1/TPM1	+	+	−

Diploids were generated and tested for growth at different temperatures on YPD. Growth rate was scored at each temperature after incubation for the following times: 37°, ≥24–48 hr; 25°, ≥2–3 days; 14°, ≥3–6 days. In each case, the growth rate of double heterozygotes was compared to the growth rate of single heterozygotes. + and − growth rate designations are similar to that described in Table 2.

[a] Double heterozygotes of all pairwise combinations of the five conditional-lethal *anc* mutations (*ancX/ANCX ancY/ANCY*) and pairwise combinations of the five *anc* mutations and mutations in the actin-binding protein genes (*anc/ANC abp/ABP*, where *ABP* denotes *SAC6, ABP1*, or *TPM1*) were constructed and tested for growth. Except for the diploids indicated in Table 4, all other combinations of mutations complemented each other at the nonpermissive temperatures.

doubly heterozygous for mutations in two *ANC* genes or in an *ANC* gene and an actin-binding protein gene. The double mutant spores were grown at 25° since the single mutants were found to grow optimally at this temperature.

In Table 5, the phenotypes of double mutants are categorized as follows: "normal," spore colony equivalent in size to the single mutant; "slow," small colonies; and "inviable" (synthetic lethal), spores did not grow into a visible colony on plates. The genotypes of inviable spores were deduced from the genotypes of surviving spore colonies. *anc1 anc4* and *anc2 anc3* double mutants were inviable while all other combinations of *anc* mutations resulted in viable double mutants. Although half of *anc1 anc3* mutants displayed small-size spore colonies, half were normal-sized and these double mutants were therefore categorized as having the "normal" phenotype. *anc1, anc3* and *anc4* mostly exhibited normal growth in combination with *abp1*, while half of the *anc2 abp1* double mutants displayed slow growth (class "normal," Table 5). While *anc1 sac6* and *anc4 sac6* mutants displayed normal growth, *anc2 sac6* mutants exhibited both slow growth and inviability (class "slow"). The growth of *anc2 sac6* mutants was further analyzed by spotting spore colonies onto YPD plates. The growth of the double mutant cells at 25° was significantly impaired relative to the growth of the single mutant cells. These data showed that the synthetic phenotype of *anc2 sac6* mutants results from slow mitotic growth rather than a delay in germination. Finally, *anc3* was inviable in combination with *sac6*. Examples of tetrads where double mutants are slow-growing (*anc2 sac6*) or invi-

TABLE 5

Synthetic phenotype of double mutants

Genotype	Growth of double mutant spores[a]			Scorable tetrads[b]			
	Normal	Slow	Inviable[d]	PD	NPD	TT	Class[c]
anc1 anc2[e]	12	0	0	4	1	10	N
anc1 anc3	5	4	1[f]	1	1	8	N
anc1 anc4	0	0	23	3	3	17	SL
anc2 anc3	0	0	11	0	3	5	SL
anc2 anc4	15	0	0	4	3	9	N
anc3 anc4	8	0	0	1	1	6	N
anc1 abp1	7	0	1	2	2	4	N
anc1 sac6	14	0	2[g]	1	3	10	N
anc2 abp1	8	8	0	1	2	12	N
anc2 sac6	1	11	3	4	3	9	S
anc3 abp1	7	2	1	0	2	6	N
anc3 sac6	0	0	28[h]	13	6	16	SL
anc4 abp1	14	0	0	5	3	8	N
anc4 sac6	18	0	0	3	5	8	N

[a] *anc/ANC abp1::LEU2/ABP1* and *anc/ANC sac6::URA3* diploids were generated and dissected to analyze the growth of double mutants originating from a single spore colony at 25°. Growth of double mutant spore colonies was compared to growth of single mutant colonies, as shown in Figure 4. Numbers under each category indicate the number of double mutant spore colonies which exhibited that growth phenotype.

[b] Tetrad analyses were based on the segregation of *anc* and *abp* mutations or between two *anc* mutations as described in MATERIALS AND METHODS. PD, parental ditype; NPD, nonparental ditype; TT, tetratype.

[c] Classification of the phenotypes of double mutants. N, normal growth; S, slow growth; SL, synthetic lethal.

[d] Three viable, one dead spores were recovered in TT tetrads and two viable, two dead spores were recovered in NPD tetrads.

[e] The *anc1Δ1::HIS3* allele was used in all double mutant studies.

[f] Two *anc1* and four *anc3* single mutant spores were dead.

[g] One *anc1* single mutant spore was dead.

[h] Three *anc3* single mutant spores were dead.

In footnotes *f*, *g* and *h*, the genotype of inviable single mutant spores was deduced from the genotype of surviving spores. It is also possible in each of these cases that a gene conversion causes the single mutant to become inviable double mutant spores.

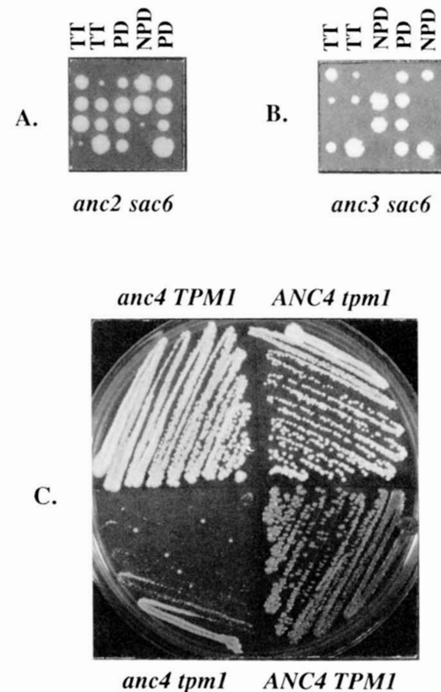


FIGURE 5.—Synthetic phenotype of *anc-abp* double mutants. (A and B) Tetrads from double heterozygotes were dissected and allowed to grow on YPD plates at 25°. Photographs were taken (after 7 days) before the genotypes of spore colonies were determined. (A) Spore colonies from *anc2-1/ANC2 sac6::URA3/SAC6* diploid (DDY400 × DDY217). Tiny colonies or inviable spores in TT and NPD tetrad types represent *anc2 sac6* double mutants (class "Slow," Table 5). (B) Spore colonies from *anc3-1/ANC3 sac6::URA3/SAC6* diploids (DDY405 × DDY217). All inviable spores in TT and NPD tetrad types are concluded to be *anc3 sac6* double mutants based on analysis of surviving spore colonies (class "Synthetic Lethal"). PD, parental ditype; NPD, nonparental ditype; TT, tetratype. (C) Tetrads from *anc4-1/ANC4 tpm1::LEU2/TPM1* diploids (DDY408 × DDY196) which were transformed with the 2μ, *URA3-TPM1*-plasmid (pDD20) were allowed to lose the plasmids as described in MATERIALS AND METHODS. Spore colonies from a tetratype tetrad that were grown on 5-FOA plates were restreaked on YPD and tested for growth at 25°. Quadrants on plates indicate *ANC4 TPM1* (DDY433), wild-type spore; *anc4 TPM1* (DDY432) and *ANC4 tpm1* (DDY431), single mutants; *anc4 tpm1* (DDY434), double mutant. The color difference of *ANC4 TPM1* cells is due to the *ade2* mutation.

able (*anc3 sac6*) are shown in Figure 5, A and B. In the case of *anc3 sac6* mutants, observations under a light microscope indicated that spores underwent anywhere from zero to about five cell divisions before arresting (data not shown). As controls for the specificity of these genetic interactions, tetrads were dissected from diploids generated by crossing *anc* mutants to *tub2-201*, *hmg1::LYS2* or *hmg2::HIS3* strains. In all cases, all tetrads were found to yield four equal-sized spore colonies (data not shown).

Synthetic interactions with *tpm1*: Similar experiments were performed to analyze the growth of *anc tpm1* double mutants. However, when tetrads from *anc/ANC tpm1/TPM1* diploids were dissected, 2:2 segregation of viable to inviable spores was observed for all *anc-tpm1* combinations (data not shown). Further analysis showed that the surviving spores were wild-type or *anc* single mutant cells, but never *tpm1* mutant cells. This phenomenon was observed for both *tpm1::LEU2* and *tpm1::URA3* alleles and depended on the presence of a mutant *anc* gene, since *tpm1* mutants crossed to a wild-type or the *hmg1::LYS2* and *hmg2::HIS3* strains produced four viable spore colonies when tetrads were dissected.

To improve the viability of *tpm1* spores, we created *anc/ANC tpm1::LEU2/TPM1* strains and transformed diploids with a 2μ, *URA3*-plasmid containing the wild-type *TPM1* gene (LIU and BRETSCHER 1989b). Wild-type strains transformed with this plasmid have been reported to contain up to 20 copies of *TPM1* and exhibit no difference in growth phenotype (LIU and BRETSCHER 1989b). These transformed diploids were then sporulated and the resulting tetrads were dissected. The presence of multicopy *TPM1* rescued the germination defect of *ANC tpm1* and *anc tpm1* spores,

TABLE 6

Genetic interactions between mutant alleles of the *ANC* and *TPM1* genes

	Growth on 5-FOA[a]						Scorable tetrads[b]		
	25°			28°					
Genotype	N[c]	S	I	N	S	I	PD	NPD	TT
anc2 tpm1	8	0	0	ND	ND	ND	4	0	8
anc3 tpm1	4	1	0	ND	ND	ND	3	0	5
anc4 tpm1	8	16	5	0	11	18	3	6	17

[a] *anc/ANC TPM1/tpm1::LEU2* diploids were generated and transformed with a 2μ, *URA3*-plasmid which contains the wild-type *TPM1*. Diploids were sporulated and their tetrads dissected. The growth of *anc tpm1* segregants was assessed by their ability to grow on 5-FOA plates (see MATERIALS AND METHODS). Numbers in each column indicate the number of double mutants exhibiting the growth phenotype. Analysis at 28° was performed only for *anc4 tpm1* colonies. ND, not determined.

[b] Tetrads were scored for the segregation of the *anc* and *tpm1::LEU2* mutations.

[c] N, normal growth (equivalent to the single mutant); S, slow growth; I, inviable.

and a high number of tetrads with four viable spores were recovered (data not shown). The *anc* mutations were identified in these spore colonies by following the Ts⁻ or Cs⁻ phenotype and the *tpm1::LEU2* allele was identified by the Leu⁺ phenotype. Growth of tetrad segregants at 25° was assayed by spotting spore colonies on 5-FOA plates to select against the *TPM1-URA3*-plasmids. Double mutant phenotypes were again scored as follows: N, normal growth; S, slow growth; and I, inviability (Table 5). The *anc2 tpm1* and *anc3 tpm1* mutants exhibited mostly normal growth. In fact, many *anc2 tpm1* double mutants grew as well as *anc2 TPM1* cells and faster than *ANC2 tpm1* single mutants on 5-FOA medium (data not shown). In contrast, most *anc4 tpm1* mutants were very slow-growing and five out of 29 independent isolates were inviable without the *TPM1*-plasmid. To avoid the slow growth phenotype of *ANC4 tpm1* cells at 25°, and to increase the synthetic phenotype penetrance of *anc4 tpm1* double mutants, we tested the same spore colonies for growth on 5-FOA plates at 28°. Although *ANC4 tpm1* haploids are temperature-sensitive, they grow better at temperatures up to 34°than at 25° (data not shown). For these reasons, 28° was found to be the optimal temperature at which to perform these experiments. As shown in Table 6, a higher percentage of inviable *anc4 tpm1* mutants was observed (62% at 28° *versus* 17% at 25°), and no double mutants were scored as having normal growth at 28°. The synthetic lethality exhibited by *anc4 tpm1* mutants is illustrated in Figure 5C. Similar experiments to analyze the growth of *anc1 tpm1* double mutants were not possible because *anc1 TPM1* mutants were found to grow poorly on 5-FOA medium.

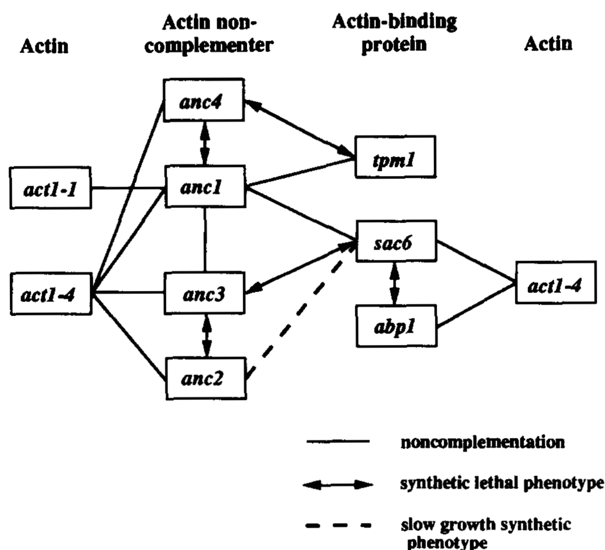


FIGURE 6.—Summary of genetic interactions. Genetic interactions between *anc* mutations, between *anc* and *act1* mutations, and between *anc* and actin-binding protein mutations are illustrated.

DISCUSSION

To identifiy new components or regulators of the actin cytoskeleton, we performed a screen for extragenic mutations that fail to complement mutant alleles of the yeast *ACT1* gene (WELCH *et al.* 1993). The experiments presented here provide strong genetic evidence that the *ANC* genes identified in these screens encode proteins that function with actin and/or actin-binding proteins in diverse ways.

Summary of genetic interactions: The genetic interactions among the *ANC*, *ABP1*, *SAC6*, *TPM1* and *ACT1* genes are summarized in Figure 6. There are multiple interactions among the *ANC* genes and between the *ANC*, *ACT1* and actin-binding protein genes. Each *anc* mutation exhibits either noncomplementation or synthetic lethality (but not both) with one other *anc* mutation, but not all pairwise combinations of *anc* mutations result in such phenotypes. Each *anc* mutation also exhibits either noncomplementation or synthetic lethality with a mutation in one actin-binding protein gene. As an exception, *anc1Δ1::HIS3* fails to complement both *sac6::URA3* and *tpm1::LEU2*. These genetic interactions occur with mutations in genes whose products associate with the actin cytoskeleton; they do not occur with mutations in *TUB2*, *HMG1* and *HMG2*. Taken together, these interactions provide strong evidence that the *ANC* gene products participate in actin functions.

In addition to the observation that these interactions were only observed with actin cytoskeletal genes, it is important to note that mutations in each *ANC* gene interact with mutations in different subsets of genes. For example, most mutations in *ANC* genes interact with mutations in either the *TPM1* or *SAC6* gene, but not both. Tropomyosin (Tpm1p) and fim-

brin (Sac6p) have distinct localizations and biochemical activities, suggesting that they are involved in different functions of actin (LIU and BRETSCHER 1989a,b; DRUBIN, MILLER and BOTSTEIN 1988). Therefore, the genetic interactions indicate that the *ANC* gene products contribute in diverse ways to actin cytoskeletal functions.

Finally, several points can be made about the types of interaction that are diagramed in Figure 6. First, the synthetic phenotypes observed in haploid cells are unlikely to be simply a consequence of additive phenotypic effects from double mutations. All of the *anc* mutants that were tested in this analysis grow well at the permissive temperature, and the disruptions of actin-binding protein genes only moderately slow the growth of cells at 25° (*SAC6*, *TPM1*), or have no effects that we have been able to identify (*ABP1*). Second, the same pair of mutant alleles never exhibited both unlinked noncomplementation and synthetic lethality, indicating that there is a fundamental difference between these types of genetic interaction. These results also imply that different genes can be identified in screens utilizing either synthetic lethality or extragenic noncomplementation. While the mechanism of extragenic noncomplementation will be discussed below (genetic models), the simplest explanation for the synthetic lethal interactions is that the mutations involved reside in genes whose products perform a similar function (BRAY and VASILIEV 1989; WITKE, SCHLEICHER and NOEGEL 1992). Alternatively, these different gene products may cooperate in order to perform a particular function (SALMINEN and NOVICK 1987; STEARNS, HOYT and BOTSTEIN 1990; KAISER and SCHEKMAN 1990; BENDER and PRINGLE 1991).

Specificity of interactions with *act1* alleles: The conditional-lethal *act1* mutations used in these studies mostly alter residues that are on or near the surface of the actin monomer. Each temperature-sensitive *act1* mutant exhibits unique genetic and phenotypic behaviors (WERTMAN, DRUBIN and BOTSTEIN 1992). Together, these observations suggest that different *act1* mutations cause defects in different interactions of actin with other actin monomers or actin-binding proteins. We tested the ability of the *anc* mutations to complement various temperature-sensitive *act1* alleles. Our results show that several classes of *anc* mutations are defined by interactions with different *act1* alleles and suggest that the *ANC* gene products interact with actin in different ways.

In general, mutations in the same domain of actin behave similarly in their noncomplementing interactions with the five conditional-lethal *anc* mutations. *act1-4*, *act1-108*, *act1-111* and *act1-112* change residues within the "large" domain of the actin monomer (KABSCH *et al.* 1990). *act1-1*, *act1-2*, *act1-122*, *act1-124* and *act1-125* change residues within the "small" domain of actin. *anc* mutations that failed to complement *act1-4* also failed to complement *act1-108*, *act1-111* and *act1-112*. Similarly, *anc* mutations that complemented *act1-1* also complemented *act1-124* and *act1-125*. As exceptions to the generalization above, the *anc1-1*, *anc1-2*, *anc3-1* and *anc4-1* mutations failed to complement *act1-2* but were able to complement *act1-124* and *act1-125*. *act1-124* (D56A and E57A) and *act1-2* (A58T) change adjacent residues, yet they behave differently in these complementation tests. These results allow us to identify differences in the functions or interactions of spatially proximal residues of actin. Finally, the different *anc* mutations also exhibit different genetic interactions with the null *act1Δ1::LEU2* allele. Taken together, the above results indicate that the noncomplementation phenotypes exhibited by the *anc* and *act1* mutations result from alterations in specific aspects of actin cytoskeletal function and do not result from additive nonspecific effects that stress the system.

Genetic models for extragenic noncomplementation: The Dosage and Poison-complex models were proposed to account for the noncomplementation of mutations in the α- and β-tubulin genes (HAYS *et al.* 1989; FULLER *et al.* 1989; STEARNS and BOTSTEIN 1988). These genes encode proteins that form heterodimers which are the subunits of microtubules. It is important to note that we modeled binary interactions between the *ANC* gene products and actin (Figure 2A), but whether such a simplification is justified awaits biochemical analyses. In the Dosage model, only one of four interactions between gene products results in a functional wild-type complex and this reduced level of functional complex cannot maintain cell viability. In the Poison-complex model, the interaction between mutant gene products results in a nonfunctional complex that interferes with normal activity. We have designed experiments to test whether the Gene-dosage or Poison-complex models can best be applied to the extragenic noncomplementation observed for different *anc* mutations. Our results support the Dosage model for some interactions and the Poison model for others.

To explore the possibility that noncomplementation results from poison-complex formation, we tested whether the noncomplementation phenotype requires the presence of the mutant actin by determining whether the *anc* mutations complement the null *act1Δ1::LEU2* allele. Mutations that lead to the formation of poison complexes might be rare compared to mutations that decrease levels of functional complexes (Dosage model, Figure 2A). Only three out of 14 *anc* mutations (*nc53*, *nc57* and *nc58*) were able to complement the null *act1Δ1::LEU2* allele but not *act1-4*, suggesting for these three mutations the possibility that noncomplementation is due to the formation of

poison protein complexes. We further tested the possibility of poison complex formation by determining whether the noncomplemention phenotypes displayed by the *nc53*, *nc57* and *nc58* mutations could be suppressed by an additional copy of the *ACT1* gene carried on a plasmid. As proposed by STEARNS and BOTSTEIN (1988), a poison complex might still exert its negative effects in the presence of excess levels of a wild-type component of the complex. However, we found that the plasmid does suppress the noncomplementation phenotype. It is possible that the failure of two mutations to complement each other is due to the formation of a poison complex, but the elevated wild-type actin competes with the mutant actin in complex formation and dilutes the poison protein complex to sublethal levels. In conclusion, the complementation of an *act1* null allele by three noncomplementing mutations suggests the existence of poison protein complexes, but more conclusive tests of this possibility must be designed.

For *anc* mutations (*e.g.*, all *anc1* alleles) that did not complement the *act1Δ1::LEU2* allele, the dosage of functional protein complex (see Figure 2A) is likely to be the parameter that is important for viability. In further support of this conclusion, the *anc1Δ1::HIS3* allele failed to complement *act1* point mutations, demonstrating that in this case the Poison-complex model can be ruled out. As an additional test of how well the Dosage model can account for the interactions between the *anc1* and *act1* mutations, we created *anc1-2/ANC1:URA3:ANC1 act1-1/ACT1* strains. The ability of an additional copy of *ANC1* to suppress the noncomplementation between *anc1-2* and *act1-1* suggests that the dosage of Anc1p is what is most important for cell viability. However, an extra copy of *ANC1* did not suppress the noncomplementation between *anc1-2* and a different *act1* allele (*act1-4*). These results can be explained by considering that the different behaviors of the two *act1* alleles might reflect a difference in the level of activity of the mutant actin proteins. *act1-1* is a recessive allele, but *act1-4* causes semidominant temperature sensitivity in an *act1-4/ACT1* heterozygote. Furthermore, the phenotype of the *act1-4/ACT1* heterozygote somewhat resembles the phenotype of the *act1Δ1::LEU2/ACT1* hemizygote (see Figure 1). Therefore, the level of functional actin might be higher in the *act1-1* background than in the *act1-4* background, explaining why the noncomplementation between *anc1-2* and *act1-1*, but not between *anc1-2* and *act1-4*, can be suppressed by an additional copy of *ANC1*. Similarly, the amount of functional Anc1p in the *anc1Δ1::HIS3/ANC1:URA3:ANC1* strain might not be equivalent to the levels expressed in an *ANC1/ANC1* diploid strain since *anc1Δ1::HIS3/ANC1:URA3:ANC1 act1-4/ACT1* diploids did not grow as well as *ANC1/ANC1 act1-4/ACT1* diploids at the restrictive temperature. An alternative explanation for why the *ANC1* duplication can suppress the noncomplementation between *anc1-2* and *act1-1*, but not between *anc1-2* and *act1-4*, is that the *act1-4* product might be more able to complex with wild-type Anc1p and remove it from the functional pool than the *act1-1* product.

Finally, the Dosage model predicts that the double hemizygote and the single hemizygotes might exhibit similar phenotypes since either could produce only 1/2 the level of functional binary complex. However, the observation that the *anc1Δ1::HIS3* allele failed to complement the *act1Δ1::LEU2* allele is inconsistent with this prediction. One possibility is that although the absolute level of functional complexes that might be formed from the two proteins is identical in either the single or double hemizygotes, the likelihood of driving the formation of these binary complexes to completion is higher in the single hemizygote because there is an excess of one type of wild-type subunit protein (*e.g.*, Anc1p in the case of *act1Δ1::LEU2/ACT1 ANC1/ANC1*).

Conclusions: The accompanying paper by WELCH *et al.* (1993) and this paper show that our screens for mutations that fail to complement *act1* mutations identified genes that are very likely to participate in actin-mediated processes. Genetic analyses indicate that these genes represent additions to the known repertoire of proteins that are important for cytoskeletal functions. Furthermore, specific interactions between mutations in the *ANC* genes and mutations in *SAC6* and *TPM1* suggest that each *ANC* gene product might participate in distinct actin functions in conjunction with a different subset of actin-binding proteins. Subsequent cloning and sequencing of the *ANC* genes, together with biochemical and cell biological studies, will provide further information about the relationships between the Anc proteins, actin, and actin-binding proteins, and about the *in vivo* roles of the *ANC* gene products.

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