Asymmetric Interactions between the Acidic P1 and P2 Proteins in the *Saccharomyces cerevisiae* Ribosomal Stalk*

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The *Saccharomyces cerevisiae* ribosomal stalk is made of five components, the 32-kDa P0 and four 12-kDa acidic proteins, P1α, P1β, P2α, and P2β. The P0 carboxyl-terminal domain is involved in the interaction with the acidic proteins and resembles their structure. Protein chimeras were constructed in which the last 112 amino acids of P0 were replaced by the sequence of each acidic protein, yielding four fusion proteins, P0-1α, P0-1β, P0-2α, and P0-2β. The chimeras were expressed in P0 conditional null mutant strains in which wild-type P0 is not present. In *S. cerevisiae* D4567, which is totally deprived of acidic proteins, the four fusion proteins can replace the wild-type P0 with little effect on cell growth. In other genetic backgrounds, the chimeras either reduce or increase cell growth because of their effect on the ribosomal stalk composition. An analysis of the stalk proteins showed that each P0 chimera is able to strongly interact with only one acidic protein. The following associations were found: P0-1αP2β, P0-1βP2α, P0-2αP1β, and P0-2βP1α. These results indicate that the four acidic proteins do not form dimers in the yeast ribosomal stalk but interact with each other forming two specific associations, P1αP2β and P1βP2α, which have different structural and functional roles.

The ribosomal stalk is an important structural element of the large ribosomal subunit directly associated with the interaction of the elongation factors during the protein synthesis elongation step in bacteria (for a review, see Ref. 1). A direct confirmation of this association has recently been shown by cryoelectron microscopy (2, 3). Involvement of the stalk components has also been reported in initiation (4, 5) and termination (6, 7).

Although the data are more scarce, in eukaryotes there is also a relationship between the stalk and the supernatant factors (8–11), which in the case of *Saccharomyces cerevisiae* EF-2 has been confirmed by electron microscopy (12). In addition to this function, the eukaryotic stalk, at least the yeast stalk, might participate in a translation regulatory mechanism not reported in bacteria (13).

The bacterial stalk is made of protein L10 and two dimers of proteins L7/L12, the amino-terminal acetylated and nonacetylated forms of a unique polypeptide. The pentamer L10-((L7/L12)_2) is extraordinarily stable (14) and binds directly to the highly conserved GTPase-related site in the 23 S rRNA (15, 16) through the L10 amino-terminal domain (17). Although the three-dimensional structure of the bacterial acidic proteins has recently been resolved (18), the detailed structure of the stalk protein complex is unknown. In fact, the ribosomal stalk is not present in the recently reported 2.3-Å resolution atomic structure of the large ribosomal subunit probably because of its high flexibility (19). Nevertheless a symmetric structure for the pentameric complex has been proposed (20), although the function of the two L7/L12 dimers in the complex might not be the same (21), and a physical separation of both dimers has been proposed (22).

The new role proposed for the ribosomal stalk in eukaryotic organisms correlates with a higher structural complexity and dynamism. Proteins P0 and P1/P2 are the eukaryotic counterparts of L10 and L7/L12, respectively. As in bacteria, one copy of P0 and four copies of P1/P2 seem to form a complex (23). However, the eukaryotic pentamer is less stable and, in contrast to the bacterial pentamer, is readily disassembled by treating the ribosome with ammonium/ethanol buffers (8). The amino acid sequence similarity of the prokaryotic and eukaryotic stalk components is rather low (24). Their structural differences are especially remarkable in the case of P0, which is larger than L10 and contains a carboxyl-end extension of around 100 amino acids not present in the bacterial polypeptide (24). This extension resembles the structure of the acidic P1/P2 proteins, containing a flexible hinge and the same highly conserved 13-amino acid terminal peptide (see Fig. 1). In fact, the P0 carboxyl extension plays the same role as the 12-kDa proteins when these acidic proteins are not present in the ribosome (25, 26). As a consequence of this functional similarity, the proteins P1/P2, in contrast to bacterial L7/L12, are not essential for ribosome activity and cell viability (27).

The acidic proteins have also evolved notably. In eukaryotes they are found as a set of genetically independent polypeptides, which can be grouped in two families, P1 and P2, and are made up of a different number of components depending on the species. Only one protein of each type has been found in mammals, insects, and fungi (28–30), whereas in protozoa (31) and yeast (32, 33) there are several. In plants, a third type of acidic protein, P3, has been reported (34).

The presence of several acidic proteins of the same type in the stalk raises a number of questions regarding their respective structural and functional roles. In *S. cerevisiae* there are four acidic proteins, two of the P1 type, P1α and P1β, and two of the P2 type, P2α and P2β. The estimation of a total of four copies of acidic proteins per yeast ribosome (35) seems to exclude the presence of one dimer of each polypeptide as reported in eukaryotes having only one acidic protein of each type, P1 and P2 (23). There is some evidence suggesting that the acidic proteins are present as monomers in the *S. cerevisiae* ribosomal

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stall (36), although this issue is still not totally settled because at least the P2 proteins are able to form dimers in solution (37). In addition, a number of questions regarding the mutual relationship between different stall components are still open.

In an attempt to explore further the structural and functional role of the P0 carboxyl-end domain as well as its interaction with the 12-kDa acidic proteins, a series of chimeras were prepared that carry a whole acidic protein replacing the last 112 amino acids forming the original P0 carboxyl terminus. These artificial constructs have been shown to be functional in yeast, and the results obtained from strains expressing them have provided relevant information on the structure of the S. cerevisiae ribosomal stalk.

**MATERIALS AND METHODS**

**Yeast and Bacterial Strains and Growth Media**

*S. cerevisiae* W303dGP0 (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP0::URA3-GAL1-RPP0) and *S. cerevisiae* D67dGP0 (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP0::URA3-GAL1-RPP0) were derived from *S. cerevisiae* W303 and D67, respectively, by integration through homologous recombination in the RPP0 locus of a construct carrying the P0 coding region fused to the GAL1 promoter (38). D45dGP0 (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP2A::URA3, RPP2B::HIS3, RPP0::kanMX4-GAL1-RPP0) and D45dGP0 (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP0::URA3, RPP0::HIS3, RPP0::kanMX4-GAL1-RPP0) were constructed in a similar way from *S. cerevisiae* D45 and D4567 (37), respectively, but using a gentamycin resistance gene as a genetic marker for the P0 gene replacement.

Yeasts were grown in either YEP medium (1% yeast extract, 2% glucose) or minimal YT medium (0.67% yeast nitrogen base, 2% glucose) supplemented with the necessary nutritional requirements. In both cases, the carbon source was either 2% glucose or 2% galactose as required. *Escherichia coli* DH5α was used for the maintenance and preparation of plasmids and was grown in LB medium.

**Enzymes and Reagents**

Restriction endonucleases were purchased from Roche Molecular Biochemicals, MBI Fermentas, New England Biolabs, and Amersham Pharmacia Biotech and were used as recommended by the suppliers. T4 DNA ligase, calf intestinal alkaline phosphatase, and the DNA polymerase I Klenow fragment were from Roche Molecular Biochemicals. DNA manipulations were performed basically as described previously (39). Polymerase chain reaction was carried out using Pfu DNA polymerase from Stratagene and custom-made oligonucleotides from Isogen following the recommendations of Diefenbach and Dveksler (40).

**Cell Transformations**

Bacterial transformations were performed according to the procedure of Hanahan (41). Yeasts were transformed using the lithium acetate method as described previously (42).

**Plasmids**

*pFL37P0/1α, pFL37P0/2α, and pFL37P0/2β*—Using appropriate oligonucleotides as primers, the genes RPP1A, RPP2A, and RPP2B encoding the acidic protein P1α, P2α, and P2β, respectively, were obtained by polymerase chain reaction using Pfu DNA polymerase from plasmids in which they were previously cloned (32, 43). At the same time, a new Nhel site was introduced downstream from the termination codon (see Fig. 1). On the other hand, taking advantage of the presence in the RPP0 gene of an EcoRV site in the position corresponding to amino acids 203 and 204 and a Nhel site in the 3′ region, the coding sequence encoding the last 112 residues was removed from RPP0 in plasmid BSP0 (38). Afterward the corresponding polymerase chain reaction fragments encoding the acidic proteins were subcloned in the same sites of BSP0 and D37. For each construct an EcoRI-XhoI fragment was excised and inserted in the corresponding plasmid constructions to give the corresponding chimeric plasmids. The chimeric genes were subsequently subcloned in the yeast expression plasmids pFL37P0/1α, pFL37P0/1β, pFL37P0/2α, and pFL37P0/2β to express the genes in yeast cells.

was removed beforehand by overlap extension polymerase chain reaction (46).

**Ribosome Extraction**

Yeasts were grown exponentially in rich YEP medium up to A450 = 0.6, and cells were collected by centrifugation and washed with buffer 1 (100 mM Tris-HCl, pH 7.4, 20 mM KCl, 12.5 mM MgCl2, 5 mM β-mercaptoethanol). Cells in buffer 1 were supplemented with protease inhibitors (0.5 μM of phenylmethylsulfonyl fluoride, 1.25 μg of leupeptin, aprotinin, and pepstatin of cells) and broken with glass beads. The extract was centrifuged in a Beckman SS-34 rotor (12,000 rpm, 15 min, 4 °C) yielding the supernatant S30 fraction, which was afterward subjected to high speed centrifugation at 90,000 rpm for 30 min at 4 °C in a Beckman TL100.3 rotor. The supernatant S100 fraction was stored at −80 °C, and the crude ribosome pellet was resuspended in buffer 2 (20 mM Tris-HCl, pH 7.4, 500 mM NaHAc, 100 mM MgCl2, 5 mM β-mercaptoethanol). When required, ribosomes were centrifuged through a discontinuous sucrose gradient (20%, 40%) in buffer 2 at 90,000 rpm for 120 min at 4 °C in a TL100.3 rotor. The pellet of washed ribosomes was dissolved in buffer 1 and stored at −20 °C.

**Protein Analysis**

Ribosomal proteins were analyzed either by 15% SDS-polyacrylamide gel electrophoresis or by isoelectrofocusing. Isoelectrofocusing was carried out as described previously (47). Particles were pretreated with RNase A (10 μg/mg of ribosomes) on ice for 30–45 min. After lyophilization, the samples were resuspended in a loading buffer (6% ampholytes, 8 μl urea) and directly loaded into a standard vertical gel (5% acrylamide, 0.2% bisacrylamide, 6 μl urea, 6% pH 2.5–5.0 ampholytes). 30 mM NaOH and 180 mM H2SO4 were used as cathode and anode solutions at the upper and lower part of the gel, respectively. Isoelectrofocusing was run in the cold room at 6-mA constant current until the voltage reached 600 V and then was lowered to 250 V for 16 h.

Proteins were usually detected by standard silver staining. Alternatively gels were stained in a solution containing 0.25% Coomassie R-250 (Sigma) dissolved in 45% ethanol, 10% acetic acid. After 30 min, the gel was destained using the same solution without stain.

**Western Blotting**

Proteins in gels were transferred to membranes, which were treated with 5% skimmed milk dissolved in TBS (10 mM Tris-HCl, pH 7.4, 200 mM NaCl) for 30 min, and afterward they were incubated for 1 h with the antibody diluted in the same buffer. Subsequently the membranes were washed for 15 min in TBS containing 5% skimmed milk and 0.1% Tween 20, and then the second antibody (Rabbit Anti Mouse or Donkey Anti Rabbit), diluted in the former buffer, was added and incubated for 30 min. Finally the membrane was washed for 15 min with 0.1% Tween 20 in TBS. Bound antibodies were detected with horseradish peroxidase-conjugated second antibodies. After silver staining or Western Blotting, the membranes were submitted to high speed centrifugation at 90,000 rpm for 30 min at 4 °C, and the crude ribosome pellet was resuspended in buffer 2 (20% Tris-HCl, pH 7.4, 500 mM NaHAc, 100 mM MgCl2, 5 mM β-mercaptoethanol). When required, ribosomes were centrifuged through a discontinuous sucrose gradient (20%, 40%) in buffer 2 at 90,000 rpm for 120 min at 4 °C in a TL100.3 rotor. The pellet of washed ribosomes was dissolved in buffer 1 and stored at −20 °C.

**Activity Tests: Polyphenylalanine Synthesis**

The reaction was performed in 50-μl samples containing 10 pmol of 80 S ribosomes, 5 μl of S100, 0.5 mg/ml RNA, 0.3 mg/ml polyuridylic acid, 40 μM [3H]phenylalanine (120 cpm/pmol), 0.5 mM GTP, 1 mM ATP, 2 mM phosphocreatine, and 40 mg/ml creatine phosphokinase in 50 mM Tris-HCl, pH 7.6, 15 mM MgCl2, 90 mM KCl, 5 mM β-mercaptoethanol. After incubation at 30 °C for 30 min, samples were precipitated with 10% trichloroacetic acid, boiled for 10 min, and filtered through glass fiber filters.

**RESULTS**

**Functionality of Protein P0 Chimeras Carrying Different Acidic Proteins as Carboxyl-terminal Domain—**Four protein P0 chimeras were constructed, P0-1α, P0-1β, P0-2α, and P0-2β in which the last 112 residues in the P0 acid sequence were replaced by each one of the acidic proteins, P1α, P1β, P2α, and P2β (Fig. 1). The overall amino acid sequence identity of the acidic proteins and the replaced P0 segment ranges from 31.5 to 45.4%, which is relatively high compared to the identity of the first 127 amino acids. A comparison of the deduced amino acid and sugar residues are excluded. In addition, an alanine- and glycine-rich region, equivalent to the hinge of the eukaryotic acidic proteins (24, 37), is found at an equivalent position in the P0 fragment (Fig. 1). The chimeric genes, subcloned in the plasmids pFL37P0/1α, pFL37P0/1β, pFL37P0/2α, and pFL37P0/2β were...
used to transform the *S. cerevisiae* P0 conditional null strains W303dGP0, D4567dGP0, D45dGP0, and D67dGP0. A summary of the strains and plasmids used is shown in Table I. In these strains, the genomic *P0* gene was replaced by a gene copy under the control of the *GAL1* promoter (38). Consequently the strains depend on the *P0* gene in the transforming plasmid to be able to grow in glucose media. As a control, the strains were also transformed with pFL37P0, which contains a wild-type copy of the *P0* gene. *S. cerevisiae* W303dGP0 contains the nuclear genes for the four acidic stalk proteins, whereas strain D4567dGP0 lacks the four genes. D67P0 and D45dGP0 lack the nuclear genes for both P1 (P1α/P1β) and both P2 (P2α/P2β) proteins, respectively (26, 27).

All the transformed strains were able to grow on glucose agar plates as well as in liquid media (Table II), indicating that the gene chimeras can complement the absence of the wild-type *P0* expression in a glucose medium. In D4567dGP0, complementation depends exclusively on the plasmid-encoded *P0* protein because there are no acidic proteins expressed in this strain (27). As expected from previous reports (27), wild-type *P0*, expressed from the control pFL37P0, allowed cell growth in glucose. In this case, replacement of the *P0* carboxyl-terminal domain by either protein P2α or P2β did not significantly affect the functionality of the resulting P0-2α and P0-2β proteins, whereas the expression of both P1 chimeras, either P0-1α or P0-1β, caused a small but clear reduction of the protein-complementing activity. In contrast, the two P0/P1 chimeras were less damaging than the P0/P2 derivatives in *S. cerevisiae* W303dGP0. In the double disruptants D45dGP0 and D67dGP0, a stimulation of cell growth occurred when the chimeras provided the acidic protein type missing in the corresponding strain. Thus, P0-1α and P0-1β stimulated growth in the D67 P1-defective cells, whereas P0-2α and P0-2β stimulated growth in the D45 strain lacking both P2 proteins.

**Composition of the Ribosomal Stalk in Cells Expressing P0 Chimeras—**Isolelectrofocusing showed that ribosomes from W303dGP0 transformed with the plasmids expressing the P0 chimeras were deprived of most of the 12-kDa proteins (Fig. 2A). Only one of them was detected in each case. Thus, proteins P2β, P2α, P1β, and P1α were found in cells expressing P0-1α, P0-1β, P0-2α, and P0-2β, respectively (Fig. 2). P1α was present in a processed form called P1α′ (49). No trace of the remaining proteins were found in the washed ribosomes. In contrast, acidic proteins accumulated free in the S100 supernatant fraction of the cells expressing the chimeras (Fig. 2B).

A similar analysis of the P2-defective D45dGP0 transformants showed that ribosomes carrying P0-2α only bound protein P1β, whereas those containing P0-2β exclusively bound P1α. The ribosomes from the same strain transformed with the P0-1α and P0-1β constructs did not contain any acidic protein in the ribosome (Fig. 3A). In the case of the P1-defective D67dGP0-derived strains the isoelectrofocusing gels showed the presence of P2β and P2α in the ribosomes from cells carrying the P0-1α and P0-1β chimeras and the absence of acidic proteins in the particles having P0-2α and P0-2β (Fig. 3B).

Therefore, regardless of the yeast strain transformed with each P0 chimera, the same associations were always detected in the batch of all cases (38). The P0-1α-P0-1β pair was associated with the particles having P0-2α and P0-2β.

**Effect of P0 Chimeras in Growth at 37 °C—**Alterations in the ribosomal stalk have previously been shown to induce some specific phenotypes when grown at 37 °C (47, 50). In this study, when the W303dGP0 transformants were grown at this temperature, the functionality of all P0 chimeras was clearly reduced, whereas D45dGP0 and D67dGP0 were unable to grow even when expressing a wild-type P0. However, growth was rescued in all cases by the presence of a moderately high salt concentration, which has no effect at 30 °C (Fig. 5).

An interesting singularity was the P0-1β-transformed...
Table II  

Growth of S. cerevisiae strains transformed with protein P0 chimeras

| Plasmid-encoded protein | Doubling time of the transformed strains (min) |  |
|-------------------------|----------------------------------------------|--|
|                         | W303dGP0 (P1α, P1β, P2α, P2β)* | D67dGP0 (P2α, P2β)* | D45dGP0 (P1α, P1β)* | D4567dGP0 (none)* |
| P0                      | 100                                       | 170                      | 231                      | 172                      |
| P0-1α                   | 125                                       | 150                      | 233                      | 196                      |
| P0-1β                   | 123                                       | 136                      | 231                      | 192                      |
| P0-2α                   | 160                                       | 212                      | 200                      | 177                      |
| P0-2β                   | 160                                       | 215                      | 208                      | 173                      |

* Ribosomal stalk proteins expressed from the genomic genes.

(bacterial stalk proteins, mainly L7/L12, interact with the factors in a very dynamic way, displaying different conformations during the elongation process (51, 52). A similar situation seems to occur in eukaryotes (12).

In contrast to the bacterial stalk components L10 and L7/L12, the carboxyl-end domain is highly conserved in the eukaryotic stalk proteins P0 and P1/P2 (13). In fact, the 100-amino acid long carboxyl-terminal domain of P0 and the P1/P2 proteins plays a similar role. Because of this functional equivalence, the acidic proteins are not essential for ribosome function but modulate its activity (36). In the absence of P1/P2, the whole P0 protein is required for cell viability, and a truncation of its carboxyl end leaving only the amino-terminal domain totally inactivates the ribosome (25). The results in this report indicate that this 100-amino acid...
were serially diluted by a factor of 10. Aliquots (5 µl) of each dilution were applied to the plates and grown at the indicated conditions and temperature.

**Table III**

| Ribosomes from strain | Phenylalanine polymerized at 30 °C | Phenylalanine polymerized at 37 °C | 37 °C/30 °C ratio |
|-----------------------|-----------------------------------|-----------------------------------|-------------------|
| W303                  | 25.5                              | 16.9                              | 0.66              |
| D67dGP0/P1α           | 17.0                              | 8.3                               | 0.48              |
| D67dGP0/P1β           | 18.6                              | 13.8                              | 0.74              |

**Fig. 5. Growth of *S. cerevisiae* strains expressing different P0 chimeras.** Cells were grown up to $A_600 = 1$ at 30 °C, and the cultures were serially diluted by a factor of 10. Aliquots (5 µl) of each dilution were applied to the plates and grown at the indicated conditions and temperature.

**Fig. 6. Acidic proteins in ribosomes from *S. cerevisiae* D67dGP0, D67dGP0/P1α, and D67dGP0/P1β grown at 37 °C.** Strains were grown at 30 °C and then shifted to 37 °C for 15 h. Ribosomes were resolved by SDS-polyacrylamide gel electrophoresis, and the proteins were detected by Western blot using specific monoclonal antibodies to either P2α (A) or P2α (B). As a positive control, ribosomes from the parental *S. cerevisiae* W303 were included (wt).

**Fig. 7. Assembly of the yeast ribosomal stalk.** Models of two possible modes of interaction of the two heterodimers P1α/P2β and P1β/P2α with P0 to form the yeast stalk. The relative position of the P1 binding site in protein P0 will determine which of the two assembly processes actually takes place.

The stalk composition seems to have not only quantitative but also qualitative effects on the translation process (27). Most D45dGP0 and D67dGP0 transformants show a temperature-related osmosensitive phenotype. However, the different growth capacity of *S. cerevisiae* D67P0/β and D67P0/α at 37 °C is significant. These data confirm that the qualitative composition of the stalk and not only the number of bound acidic proteins determine the capacity of the cell to grow in certain conditions. This differential growth seems not to be due to important differences in the overall efficiency of their respective translation machineries (Table III) but probably to the specific effect on the synthesis of some proteins by ribosomes carrying a particular stalk composition. Similar phenotypes have been found in other ribosomal stalk mutants (47, 50). We are presently exploring whether or not a specific component in the cellular cell wall integrity signal transduction route is affected in these strains. So far no significant alterations in the dual phosphorylation of the mitogen-activated protein kinase Slt2 kinase, which is usually activated in cell wall-defective strains (53), have been detected in our mutants. Other components of the same route are being tested in the hope of identifying proteins whose expression is affected by the ribosomal stalk composition.

The most interesting conclusions from this report are probably those concerning the overall stalk structure. The presence of four acidic proteins makes the yeast ribosomal stalk different from bacteria and even other eukaryotes, raising interesting structural questions with evident functional implications. An obvious issue is the interaction among the different stalk components. The bacterial counterpart, protein L7/L12, has been shown to be present as dimers in the ribosome (1) as well as P1 and P2 in some eukaryotes (23). However, the results indicate that it is not the case in *S. cerevisiae*. Thus, yeast ribosomes carrying a P0 chimera only bound one 12-kDa acidic protein, and this was never the one that was fused to the chimeric P0 (Fig. 5). The most direct conclusion from these data is that none of the yeast 12-kDa proteins is able to self-associate to form dimers.

1. M. Molina, M. Remacha, and J. P. G. Ballesta, unpublished results.
dimers in the ribosome. They are present as monomers, indicating that contrary to the bacterial proteins they do not need to be dimerized to be functional. Nevertheless, the yeast acidic proteins do not function independently. Our results clearly show that there is a favored association between P1α with P2β on the one hand and P1β with P2α on the other. These two protein pairs might be considered as heterodimers playing the role that the two L7/L12 dimers do in bacteria.

Data pointing to a different structural and functional role for the P1α/P2β and P1β/P2α pairs were reported earlier. Thus, both protein couples are differentially affected by the absence of protein L12, which interacts with the GTPase rRNA near the P0-P1/P2 binding site (54). A similar situation was found when *S. cerevisiae* P0 was replaced by the equivalent protein from other species (45). Moreover, the two acidic couples have a differential effect on the resistance of the ribosome to some translocation inhibitors (55). Physicochemical studies have indicated an interaction between P1α and P2β in solution (56), and it was also recently reported that protein P2α but not P2β protects P1β from degradation in the cell, confirming the specificity of the P1β/P2α association (57).

The available data indicate that the wild-type yeast ribosomal stalk is formed by the interaction with protein P0 of two acidic protein complexes, P1α-P2β and P1β-P2α, which already seem to be present in the cell cytoplasm (57). Protein P0 seems to be able to directly interact only with the P1 proteins as shown previously by ribosome reconstitution tests (58) and double hybrid data (36), and it is possible, therefore, that a direct interaction between P0 and the P2 proteins may not exist in the ribosome. There are at least two ways in which P1α·P2β and P1β·P2α can be assembled in the stalk (Fig. 7). In one of them the two proteins of the same type are adjacent at one of the ends. In the other, the four proteins alternate in such a way that the proteins of the same type can hardly touch because they are separated by a protein of the opposite type. There is not enough experimental information to decide which of the possible models is correct, and a detailed study of the interaction among all the different stalk components using different experimental approaches is being carried out in an attempt to resolve this question.

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