Actin can exist in multiple conformations necessary for normal function. Actin isoforms, although highly conserved in sequence, exhibit different biochemical properties and cellular roles. We used amide proton hydrogen/deuterium (HD) exchange detected by mass spectrometry to analyze conformational differences between *Saccharomyces cerevisiae* and muscle actins in the G and F forms to gain insight into these differences. We also utilized HD exchange to study interdomain and allosteric communication in yeast-muscle hybrid actins to better understand the conformational dynamics of actin. Areas showing differences in HD exchange between G- and F-actins are areas of intermonomer contacts, consistent with the current filament models. Our results showed greater exchange for yeast G-actin compared with muscle actin in the barbed end pivot region and areas in subdomains 1 and 2 and for F-actin in monomer-monomer contact areas. These results suggest greater flexibility of the yeast actin monomer and filament compared with muscle actin. For hybrid G-actins, the muscle-like and yeastlike parts of the molecule generally showed exchange characteristics resembling their parent actins. A few exceptions were a peptide on top of subdomain 2 and the pivot region between subdomains 1 and 3 with muscle actin-like exchange characteristics although the areas were yeastlike. These results demonstrate that there is cross-talk between subdomains 1 and 2 and the large and small domains. Hybrid F-actin data showing greater exchange compared with both yeast and muscle actins are consistent with mismatched yeast-muscle interfaces resulting in decreased stability of the hybrid filament contacts.

The ability of actin to engage in a wide range of physiological functions requires that it be subject to complex spatial and temporal control by a large array of actin-binding proteins (1–3). Such regulation demands that both the monomeric and filamentous forms of actin be able to exist in a number of different conformations. Some of these may be differentially recognized by different actin-binding proteins, and some might actually be induced by the interaction of actin with these proteins.

Actin is highly conserved from yeast to humans. There is 87% sequence identity between yeast and muscle actin and 91% sequence identity between yeast and nonmuscle actins. Even with this high sequence similarity and minor differences in crystal structures, there are some major differences in yeast and muscle actin behavior. Yeast, compared with muscle actin, polymerizes faster and exchanges its bound nucleotide faster. In contrast to muscle actin, the yeast actin filament releases its P, almost immediately after ATP hydrolysis and the filament fragments more readily (4). Yeast cells cannot survive with muscle actin as the sole actin, and with β-nonmuscle actin as the only actin, yeast cells are very sick (5). A set of biochemical data indicates that the muscle filament is less flexible than yeast (6, 7). However, conformational and structural differences that could explain these behavioral differences are unknown.

The actin molecule is divided into two domains, the small domain consisting of subdomains 1 and 2 and the large domain consisting of subdomains 3 and 4. Modeling studies (8, 9) have suggested that each of the subdomains can move, and biochemical studies have suggested allosteric interactions between the subdomains. However, the nature of these interactions has not been elucidated on a molecular level.

To try to gain insight into what parts of the actin molecule are important for the behavioral differences observed between yeast and muscle actin, yeast-muscle hybrid actins were constructed (10). Hybrid sub1 actin has muscle-specific residues introduced into the small domain of actin, making it muscle-like in subdomain 1 and yeastlike in subdomains 2, 3, and 4. Sub12 hybrid actin has muscle-specific residues in both subdomains 1 and 2, making it muscle-like in subdomains 1 and 2 and yeastlike in subdomains 3 and 4. These actins showed muscle-like behavior in several biochemical properties: nucleotide exchange rates, thermostability, and the nucleation phase during polymerization. Although most of the residues proposed to be involved in the interaction of actin with myosin are located in subdomains 1 and 2, both hybrids exhibited yeast actin behavior in the activation of myosin ATPase activity. The hybrid actins also exhibited higher critical concentrations than either yeast or muscle actin and formed ADP-F-actin that was less stable than either yeast or muscle actin (10).

The experimental results with the hybrid actins indicated that the behavior of actin as a whole is dictated by the interaction and cross-talk of the two halves. One experimental approach to further understand the structural bases of 1) biochemical differences observed with yeast and muscle actins, 2) how introduction of muscle-specific residues affects conformational behavior of the hybrids, and 3) whether there is propagation of change and cross-talk from muscle domains into yeast domains of the hybrid actins in solution is to use NMR. How-

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S9.
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Actin Isoform Conformational Differences

However, NMR as a technique is not suitable here due to the high actin concentrations that would be needed and the propensity of actin to aggregate at these concentrations. Therefore, we utilized hydrogen-deuterium (HD)\(^2\) exchange detected with mass spectrometry. This is a powerful technique that can be used to obtain information about conformational changes in proteins in solution on a global as well as a local level.

A protein sample placed in a deuterated solution will exchange its amide protons of the polypeptide backbone with exchange times varying from seconds to months (11). The amount and rate of deuterium exchange will depend on factors like temperature, pD, accessible surface area, local environment, and conformational flexibility (12–15). Labeled protein samples are digested by pepsin, the protease of choice in HD experiments, because of the requirement for acidic pH in order to minimize back exchange. The resulting peptide fragments are separated by high pressure liquid chromatography and analyzed for deuterium uptake.

For a peptide that is undergoing amide proton exchange, the average mass of the peptide will increase with labeling time with an increase in mass greater for the peptides that are more exposed to the solvent. The analysis of deuterium uptake for specific peptides and areas of proteins could potentially provide information about structural and conformational differences between different actin forms. Since changes in conformation among different actin states will affect deuterium uptake, HD exchange coupled with mass spectrometry should provide us with a tool to study the states of actin present in solution. HD exchange detected with mass spectrometry was previously used successfully by Chik and co-workers (16) to assess changes in muscle actin structure due to the G- to F-actin transition and the binding of phallodin to F-actin and of DNase I to G-actin. More recently, the technique has been used to assess conformational changes in the Arp2/3 complex, which contains actin.

Hydrogen-Deuterium Exchange—A protein sample in the appropriate buffer was diluted in ~16 times its volume of deuterated buffer. For F-actin samples, concentrations of actin were about 2 or more times critical concentration. Critical concentrations for all actin isoforms used are comparable. Exchange was carried out at room temperature at a pH of 7.5. After certain labeling times, aliquots of ~300 pmol of protein were taken, and the exchange reaction was quenched by putting the sample in 100 mM phosphate buffer, pH 2.5, and then by freezing the sample on dry ice. Samples were stored at ~80 °C and analyzed by mass spectrometry in the next 2 days.

Mass Spectrometry Analysis—Labeled protein samples were analyzed as described previously (20). Briefly, labeled protein samples were injected into a system connected to a pepsin column (21). The samples were loaded on the pepsin column with a flow rate of 100 μl/min and, after digestion, loaded on a peptide macrotrap (Michrom Bioresources, Inc.) for desalting. Pepsin digestion and desalting on the macrotrap lasted 6 min with a digestion time on the pepsin column of less than 2 min. The peptides generated were loaded on a Jupiter Proteo C-12 column and eluted in a mass spectrometry instrument with an acetonitrile gradient from 15 to 85% (v/v) in 6 min at a flow rate of 50 μl/min. To eliminate back-exchange, the system with the injectors, peptide macrotrap, C-12 column, and accompanying tubing was immersed in an ice-water bath. The pepsin column was above the ice bath. The mass spectrometer used was an LCQ Deca (Thermo Finnigan) in the University of Iowa High Resolution Mass Spectrometry Facility.

The mass spectra obtained were analyzed with the Bioworks Browser and Xcalibur Qual Browser (Thermo Finnigan) to identify the peptides, and the centroid mass of the peaks was determined with MagTran (22). Data were not adjusted for back-exchange, and therefore all changes in masses were reported as uncorrected changes.

EXPERIMENTAL PROCEDURES

Materials—DNase I (grade D) was purchased from Whatman. Rabbit muscle acetone powder was obtained from Pel-Freez. Porcine pepsin was obtained from Sigma. All other chemicals used were of reagent grade quality.

Protein Purification—Wild type Saccharomyces cerevisiae was obtained from a local bakery. Wild type actin from S. cerevisiae and hybrid actins from appropriate strains were purified using DNase I affinity chromatography and DE52 DEAE anion exchange chromatography according to Cook et al. (18). The actin was then subjected to a round of polymerization-depolymerization. Muscle actin was purified from rabbit muscle acetone powder, as described by Spudich and Watt (19). The purified actins in their calcium form were stored at 4 °C in G-buffer (10 mM Tris-HCl, pH 7.5, 0.2 mM CaCl\(_2\), 0.2 mM ATP) and used within 4 days. To obtain F-actin, polymerization was induced by the addition of 2 mM MgCl\(_2\) and 50 mM KCl to a G-actin sample at room temperature.

RESULTS

Deuterium Uptake for Yeast G- and F-actins—To examine possible structural and conformational differences between different actin isoforms, we used HD exchange as described above. Good coverage of the actin sequence was achieved with pepsin digestion and subsequent separation steps. For all of the actin isoforms studied, yeast, muscle, sub1, and sub12, depending on the run and the isoform, between 40 and 50 peptides were usually seen, which covers about 82–92% of the sequence. Supplemental Figs. S1–S4 show the yeast, muscle, sub1, and sub12 actin sequences and peptides obtained following pepsin digestion and mass spectrometry analysis. One of the drawbacks of the experimental setup for HD exchange detected with mass spectrometry is that peptide detection can be somewhat stochastic and will not be identical for each run. The result is that some peptides might not be seen in every run. Fig. 1 shows mass spectra of a representative yeast G-actin peptide and its increase in mass with labeling time. The peptide 105–121 has 14 exchangeable amide hydrogens and is a part of a helix and a

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2 The abbreviation used is: HD, hydrogen/deuterium.
β sheet located in the back of subdomain 1. For all the samples, labeling times were from 30 s to 3 h.

Fig. 2 shows deuterium uptake for a 6-min labeling time for peptides of yeast G- and F-actin. Peptides are divided into several groups based on their change in mass with labeling time calculated as follows,

\[
\text{% exchange of peptide amide hydrogens} = \left(\frac{m_l - m_u}{n}\right) \times 100 \quad \text{(Eq. 1)}
\]

where \(m_l\) is the mass of the labeled peptide, \(m_u\) is the mass of the unlabeled peptide, and \(n\) is the number of exchangeable amide hydrogens, which is equal to number of peptide bonds minus the number of prolines minus 1. Thus, the percentage exchange of peptide amide hydrogens depicted in our graphs is the change in mass of the total peptide due to deuterium incorporation normalized by the total number of exchangeable amide hydrogens. After 6 min of labeling, most of the molecule shows deuterium uptake of 20–40% for yeast G-actin. Areas that exchange the most (40–60% of their hydrogens exchanged) are located in the bottom of subdomain 1 (peptide residues 347–355, 58% exchanged, one run), top of subdomain 2 (peptide 32–44, 53 ± 2% exchanged; peptide 45–66, 53 ± 15% exchanged), and a β sheet in subdomain 4 (peptide 222–235, 42% exchanged, one run) that is largely exposed to the solvent. Areas that exhibit the least HD exchange (0–20%) are located throughout the molecule. These include areas that are buried from the solvent, such as the peptide near the N terminus in subdomain 1 (peptide 8–16, 16 ± 1% exchanged), and a β sheet in subdomain 3 (peptide 154–168, 18 ± 2% exchanged). On the other hand, some of the peptides that exhibit very little exchange after 6 min of labeling time are somewhat more exposed to the solvent based on the actin crystal structure. These peptides include part of a helix in subdomain 1 (peptide 83–94, 9 ± 1% exchanged) and two other helices in subdomain 4 (peptide 206–218, 9 ± 3% exchanged; peptide 177–189, 9 ± 2% exchanged).

In F-actin, monomers come together to form a double-stranded filament. According to the Holmes actin filament model, subdomains 3 and 4 largely constitute the inside surface of the filament, whereas subdomains 1 and 2 make up the outside filament surface (23, 24). Monomer-monomer contacts in the filament include a contact between the top of subdomain 2 of one monomer and the bottom of subdomain 3 of a second. Furthermore, the C terminus and top of subdomain 4 make contacts with the bottom of subdomain 3 of another monomer. Considering the protection from the solvent due to the contacts made between the monomers in one strand of the filament and also between the strands, less HD exchange should be seen with the filament compared with the monomer. Indeed, this is the case, and yeast F-actin incorporates about 13 fewer deuteriums per monomer than G-actin, observed throughout the molecule. As seen in Fig. 2, after 6 min of labeling, peptide 236–261 in subdomain 4 of F-actin exchanges 19 ± 1% of its exchangeable amide hydrogens compared with 30 ± 3% in

![FIGURE 1. Increase in mass with labeling time for a representative peptide of yeast G-actin. The peptide in question is 105–121 and has 14 exchangeable amide hydrogens. Top, unlabeled sample; Second spectrum, 30-s labeling time; Third spectrum, 6-min labeling time; Bottom, 3-h labeling time.](image-url)
G-actin. Also, the C terminus peptide 356–375 in subdomain 1 shows 18 ± 4% exchange compared with 25 ± 2% in G-actin. Two more peptides that exhibit exchange levels that were lower for F- compared with G-actin were peptides 268–283 with exchange levels of 26 ± 3% for G-actin and 19 ± 2% for F-actin and peptide 284–299 with exchange levels of 12 ± 2% for G-actin and 29 ± 3% for F-actin. Interestingly, peptide 326–340, located in subdomains 1 and 3 connecting the two subdomains and the two halves of the actin molecule, exchanged 42 ± 3% of its exchangeable amide hydrogens compared with a 37 ± 2% exchange in G-actin. This peptide is the only peptide observed that showed significant increase in exchange in F- compared with G-actin.

A more sensitive way to examine the differences between G- and F-actin is to observe exchange within a peptide over multiple time points. Below, peptides referred to are depicted graphically in the figures along with examples of the kinetic data. The remainder of the kinetic data are included in the supplemental material. Supplemental Figs. S3, S5, and S6 show HD exchange with time for peptides that exhibited significant differences in exchange between yeast G- and F-actin. Graphs shown are averages and the ranges of HD experiments performed with two different preparations of actins or averages with S.D. values for HD experiments done with three or more different actin preparations. Specific peptides were judged as showing significant differences in exchange between different forms of actin if the average exchange levels and S.D. values for different forms of actin did not show overlap. Also, the difference was deemed significant if there was a trend exhibited throughout the labeling time. Data presented show change in HD exchange with labeling time for the first 10 min of labeling. For most of the peptide pairs with distinguishable differences, the peptide exchange curves seemed to show more difference in total protons exchanged (intercept) rather than differences in the rates of exchange over time. However, because of nonlinearity in these plots and small apparent differences in slopes if any, we did not actually calculate slope values.

For most of the peptides, for labeling times longer than 10 min, there is less of a difference observed between different actin forms. In the nine peptides represented, eight peptides show decreased exchange in F-actin compared with G-actin, and one peptide showed increased exchange in F- versus G-actin. Areas of decreased deuterium exchange in F- versus G-actin include areas that were predicted contacts in the Holmes filament model. These consist of peptides 32–44 and 45–65 of subdomain 2 (supplemental Fig. S5), peptide 356–375 in the C terminus (supplemental Fig. S6), peptide 206–218 in the subdomain 4, and peptides 236–261 (supplemental Fig. S6) and 262–269 in subdomain 4 (Fig. 3). Peptide 105–121 also showed a decrease in exchange in F- compared with G-actin (Fig. 3). This area was not predicted to be involved in monomer–monomer contacts in the Holmes model. However, a segment of this peptide was predicted to make monomer–monomer contacts in a recently published model of the filament by Oda et al. (25). The position of this peptide in the context of monomer–monomer contacts in the Oda filament trimer is represented in Fig. 4. Fig. 4 also represents other peptides that show differences in exchange between F- and G-actins and their position in the Oda filament trimer. According to the Holmes or Oda filament models, peptide 95–104 in subdomain 1 should not be masked by involvement in a monomer–monomer interface. Therefore, the G- to F-actin transformation should not decrease the exchange. However, it actually showed less of an exchange in F- versus G-actin (supplemental Fig. 5). On the other hand, peptide 326–340 located between subdomains 1 and 3 showed increased exchange for F-actin for early labeling times (Fig. 3). As mentioned earlier, this is the only peptide that showed increased exchange in F- versus G-actin.

Differences in HD Exchange between Yeast and Muscle G-actins—Deuterium uptake was compared for yeast and muscle G-actins to try to elucidate conformational differences that could explain behavioral differences seen between the two actin isoforms. Peptides that we compared are located in the same area of the molecule and have the same number of exchangeable amide hydrogens, although they might have differences in amino acid sequence and in their masses. Peptides that show significant differences between yeast and muscle G-actins are peptides 17–31 and 105–121 located in subdomain 1, peptide 32–44 in subdomain 2, and peptide 143–153 in subdomain 3. In all four cases, the peptide in yeast actin exchanges more than...
Interestingly, three of four peptides are located in the smaller domain of the actin molecule. The fourth peptide is located after the hinge helix of subdomain 1 and makes a part of the helix and β sheet that connect the two halves of the protein.

Differences in HD Exchange between Yeast and Muscle F-actins—Deuterium uptake was compared for yeast and muscle F-actins. As was the case for G-actins, equivalent peptides were compared. Differences are observed with peptides in subdomain 2 peptides 32–44 and 45–65 and in subdomain 4 peptide 236–253. Both areas involve regions where proposed monomer-monomer contacts are made in the Holmes model. In all three peptides, yeast actin exhibits greater exchange compared with muscle actin (Fig. 6 and supplemental Fig. 7). Another peptide that shows greater exchange for yeast compared with muscle actin is peptide 105–121 (Fig. 6). As mentioned previously, amino acids within this peptide are involved in monomer-monomer contacts in the filament model of Oda et al. (25).

Differences in HD Exchange between Yeast, Muscle, Sub1, and Sub12 G-actins—in our previously constructed yeast-muscle hybrid actins, subdomains 3 and 4, are completely from yeast actin, and sub1 and sub12 refer to the subdomains carrying the entire complement of muscle specific residues (10). If the two halves of actin are allosterically coupled across the nucleotide cleft, the creation of yeast-muscle hybrids might create mismatches resulting in altered interdomain communication. Such a result might be manifested as an alteration in peptide HD exchange patterns. We thus monitored HD exchange for the two hybrid actins. Sub1 and sub12 G-actins show differences in HD exchange compared with yeast G-actin in several peptides located mostly in the smaller (muscle-like) actin domain (Fig. 7 and supplemental Fig. S8). These peptides include 17–31, 83–94, 95–104 (supplemental Fig. S8), and 105–123 in subdomain 1 (Fig. 7), which, as in muscle actin alone, exchange to a lesser extent than in yeast actin. The behavior of peptide 32–44 in subdomain 2 was surprising. This peptide differs between yeast and muscle actin at one position; at position 43, there is a valine in yeast actin compared with isoleucine in muscle actin. Its exchange levels were low, like
muscle actin, regardless of whether or not muscle residues are in subdomain 2 (Fig. 7). Another peptide showing a difference in exchange is peptide 143–153 located between subdomains 1 and 3 expanding from the so-called hinge region (8). In both hybrids, although the sequence is that of yeast actin, the peptide exhibits exchange behavior similar to that of muscle actin (Fig. 7).

Differences in HD Exchange between Yeast, Muscle, Sub1, and Sub12 F-actins—In the filament, a different type of result was observed. In most of the peptides for which significant differences in exchange occurred, sub1 and sub12 peptides showed higher extents of exchange compared with muscle F-actin (Fig. 8 and supplemental Fig. S9). These peptides include those thought to be involved in monomer-monomer contacts in the filament: peptides 32–44 for both sub1 and sub12 and peptide 45–65 for sub1 in subdomain 2 (Fig. 8 and supplemental Fig. S9). Peptides 206–218 and 200–218 in subdomain 4 also showed higher exchange levels compared with muscle F-actins (supplemental Fig. 9). Another peptide of interest is peptide 286–299 located on the bottom of subdomain 3. This peptide, observed in yeast, sub1, and sub12 actin analyses, exhibited increased exchange levels for both hybrids compared with yeast F-actin (supplemental Fig. S9). Peptide 105–121 in subdomain 2 also showed increased levels of exchange compared with muscle F-actin for both hybrids (Fig. 8). Differences were also observed with peptide 68–84 in the area in subdomain 2 involved in ATP binding. This region also contains a histidine at position 73, which is methylated in muscle actin but not in yeast nor in either of the two hybrids, most likely due to the lack of the methylase in yeast (26). Peptide 68–84 in subdomain 1 showed increased levels of exchange compared with muscle actin for the sub12 hybrid (Fig. 8). The equivalent peptide was not detected in the yeast actin analysis, and therefore a comparison with yeast actin could not be performed.

DISCUSSION

The focus of this work was to use mass spectroscopic analysis of HD exchange to examine at three different levels the role that conformational differences play in regulation of actin biological function. First, we wished to gain insight into the different conformations assumed by the monomeric versus the filament form of a single actin species. Second, we wished to explore the extent to which conformational differences between different actin isoforms might underlie their different physiological roles. Finally, we wished to gain insight into the allosteric communication within actin that controls the interconversion of these different conformations.

G to F Transition of Yeast Actin—Incorporation of an actin monomer into a filament should sequester those surfaces involved in monomer-monomer contacts or, alternatively, decrease the mobility of peptides very near these contacts. Either case would lead to protection from HD exchange. Our results generally agree with this hypothesis. Seven peptides whose rates of exchange are slowed following incorporation into F-actin clearly fall in or very near a proposed site of inter-monomer contact according to the Holmes filament model. These include the top of subdomain 2, the top of subdomain 4, and, interestingly, the top of the hydrophobic plug that reaches up along the side of the subdomain 4 surface from the subdomain 3/4 boundary.

The original Holmes model predicted that in formation of the filament, the hydrophobic loop consisting of residues 262–274 would extend from its parked position along the filament so that it inserted into a hydrophobic pocket made up of the interface of two monomers in the opposing strand of the filament. Extensive investigation found no evidence in support of this
extension (27, 28). However, based on fluorescence studies and cross-linking experiments, it was shown that this loop had to undergo a conformational change to another parked position for polymerization to occur (28–30). Our data support this change and further suggest that in the polymer state, some or all of the amide protons of peptide 262–269 encompassing the tip of this hydrophobic plug are in a more sequestered or immobile state.

Unexpectedly, based on the Holmes model, peptide 105–121 also exhibited decreased exchange. In the original model, this was not part of or near an interface. However, Oda et al. (25) recently published a variation of this model showing a 20° rotation of subdomain 2 not present in the original structure. This was caused by a bend in the polypeptide at 141–142 and 336–337 that would place a segment of peptide 262–269 encompassing the tip of this hydrophobic plug are in a more sequestered or immobile state.

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The pivot region at the barbed end of the monomer connecting both halves of the protein is an important factor in establishing the degree of movement of the two full domains relative to one another in the form of shearing or scissors-like movements. The extent of these movements will directly translate into changes in filament dynamics and flexibility. We observed increased exchange of peptide 326–340 in the filament compared with the monomer in yeast actin, whereas no such difference was observed with muscle actin in a previous study by Chik et al. (16). Since, as discussed earlier, this peptide is in the creation of monomer-monomer interfaces. Therefore, these data are more consistent with an induced fit instead of a more lock-and-key polymerization mechanism, terms usually applied to descriptions of enzyme mechanisms.

The G to F transition of muscle actin was previously studied by HD exchange and mass spectrometry by Chik et al. (16). Results of that study are in general agreement with our results. Peptides that showed the same trend in the G to F transition of muscle actin (i.e. protection from the solvent in F-actin) showed the same trend in our study of yeast actin G to F transition. The only exception is peptide 326–340, discussed below.

**Comparison of Yeast Versus Muscle Actins**—Our HD exchange results allowed us to gain insight into the biochemical differences between yeast and muscle actins that translate into functional differences in the cell despite their high degree of sequence conservation. For all peptides that could be compared between the two actins, where differences existed, the yeast peptide exchanged more than its muscle counterpart in both G- and F-actin. For F-actin, these differences were observed in or near areas involved in monomer-monomer contacts within the filament model. This result correlates with the observation that yeast actin fragments to a greater extent than does muscle actin (4), perhaps resulting from less stable or more flexible monomer-monomer interfaces that are manifested by increased exposure to solvent. A similar association between increased filament flexibility and increased tendency to filament fragmentation was also made in a study of the binding of cofilin to F-actin (30). The results are also consistent with the work done previously with electron microscopy and three-dimensional reconstruction showing that the yeast actin filament has a nucleotide binding cleft that appears more open than that in the muscle filament and that the yeast filament has less extensive contacts between the two long pitch strands (31).

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pivot point region, this difference is consistent with greater twisting and perhaps greater flexibility of the monomer within the filament, in line with the apparently more dynamic behavior of yeast F-actin. We also observe isoform differences between yeast and muscle actin in subdomain 3 peptide 143–153, one of two regions connecting the two domains near the pivot region and part of the shear region defined by Page et al. (9). The increased rate of exchange of the yeast versus muscle peptide again is consistent with greater solvent exposure and perhaps greater propensity of the yeast monomer to open and twist. This area is also involved in the interaction of actin with binding proteins, such as cofilin (32), and WH2 domain proteins, such as formin (33), which are involved in controlling actin filament polymerization and turnover. Different flexibility and accessibility of this region to the solvent could be important for actin isoform specificity in the interaction of actin with its binding partners.

Another peptide that potentially can influence both monomer and filament behavior of the actin is peptide 105–121 in subdomain 2. It is part of a solvent-exposed α helix and a more buried β sheet that lies right below the region that contains residues 70–78, part of the sensory loop involved in regulation of the behavior of the bound nucleotide. As mentioned above, it is also involved in monomer–monomer contacts in the new Oda et al. filament model, in the binding of cofilin to the filament (32), and in the binding of profilin to the actin monomer (34). Our observation of increased exchange of the yeast relative to the muscle peptide again is consistent with the more dynamic biochemical behavior of the yeast actin filament.

Yeast actin has 91% sequence identity with nonmuscle actins, and it contains residues typical of nonmuscle versus muscle actins, such as a hydrophobic residue at position 10 and a Cys at position 17. Thus, yeast actin can actually be thought of in a broader context as a model nonmuscle actin. Several studies have reported differences in the biochemical behavior of muscle versus nonmuscle isoforms with respect to inherent filament characteristics, such as rheology, differences in cooperative ligand binding, and interaction with different actin-binding proteins (35–37). Furthermore, these differences have been linked to differences in the flexibility of muscle versus nonmuscle actin filaments. The differences in HD exchange between yeast and muscle actins for peptides involved in monomer–monomer interfaces provide a structural basis for the previously reported solution differences between muscle and nonmuscle actins in general.

Hybrid Actin Studies—One can think of the actin molecule as a clam shell with two domains connected by a hinge region between subdomains 1 and 3 at the barbed end of the protein and a nucleotide bridge across the interdomain cleft. Based on this analogy, construction of a yeast–muscle hybrid actin in which one domain contains all yeast residues and the other contains all muscle residues provides a unique opportunity to explore how these two domains influence one another. For G hybrid actins, the peptides in subdomains 1 and 2 generally exchange more slowly than yeast and much more like those from muscle actin, perhaps reflecting the muscle-specific residues in this domain. This overall result suggests that in the monomer form, there is a substantial amount of independence in the behavior of one of these half-shells relative to the other.

There were some surprises, however. The slow exchange of subdomain 2 peptide 32–44 near the pointed end of the protein was independent of whether the three subdomain 2 muscle residues were present. This result indicates that the nature of subdomain 1, muscle-like in this case, can generate propagated allosteric effects that control the pointed-end behavior of the protein. The second unexpected observation was the slow muscle-like exchange of peptide 143–153 near the pivot region in
the yeast half of the molecule in subdomain 3. Evidently, the nature of subdomain 1 can exert allosteric effects through the pivot region into this part of the protein.

Incorporation of a monomer into a filament should produce a masking of surfaces and restriction of movement, leading to an overall picture of decreased HD exchange. Yet, with the hybrids, the results produce a very different picture concerning peptides thought to be part of monomer–monomer interfaces, especially comparing sub12 and yeast actins. In the hybrid, two subdomain 2 peptides that make an interface in the Holmes model and two subdomain 1 peptides in the muscle half of the hybrid, one of which is an interface residue in the new Oda filament model, actually exchange faster than their counterparts in muscle actin. Furthermore, in the yeast half of the protein, the region encompassing subdomain 4 residues 206–218 near the surface of the interdomain cleft in the yeast half of the protein actually exchanges faster than its WT yeast counterpart, as does peptide 286–299 in subdomain 3. These results suggest that the hybrids form mismatched yeast-muscle interfaces, resulting in decreased stability of the filament contacts and consequently increased HD exchange. The hybrid filaments also resulted in increased HD exchange near the nucleotide binding pocket in the muscle-half of the protein relative to WT muscle actin, possibly resulting from increased movement at the monomer–monomer interfaces.

For hybrid F-actins, differences are observed with peptide 68–84. This region is in the area in subdomain 2 involved in ATP binding, and it also contains the so-called sensory loop, residues 70–78 (38). His$^{73}$ in the sensory loop is methylated in muscle actin but not in yeast or either of the two hybrids. This histidine residue has been suggested to play a role in establishing the stability of the filament due to its potential ability to retard the release of organic phosphate generated after ATP hydrolysis (39, 40). The difference in exchange observed with peptide 68–84 for hybrid actins could be partly due to the lack of methylation on His$^{73}$. Molecular dynamics simulation of monomeric actin with different bound nucleotides showed that in ATP and ADP-P$_i$ actin, the sensory loop area forms a $\beta$ sheet that is stabilized by a hydrogen bonding network. Conversely, in ADP actin, it is an unstructured coil that loses most of its hydrogen bonds (41). This area of the molecule is muscle-like and has muscle-specific amino acid residues important in the interaction of actin with ATP. Nevertheless, this peptide observed for the hybrid F-actins showed greater exchange compared with muscle actin. This result is consistent with this area of the protein being less structured and losing its hydrogen bonds, as seen with ADP actin.

Residues of actin involved in making the contacts with the nucleotide are located around the nucleotide binding cleft in both domains. Therefore, another possibility for the hybrid actin difference is that there is a propagated change through the nucleotide from the larger domain onto the smaller domain of actin. A mismatch between the domains and therefore a mismatch in the interaction with the nucleotide could be evident in terms of greater flexibility and exchange of these peptides in subdomain 2. The equivalent peptide was not detected in the yeast actin analysis; therefore, a direct comparison with yeast actin could not be done. This entire array of results involving monomer–monomer interactions and altered behavior of the stability-determining nucleotide pocket correlates with an increased tendency of the hybrid filaments to fragment, producing more barbed ends and a resulting faster rate of polymerization, as we observed earlier (10).

In summary, there is widespread acknowledgment that actin is a very conformationally dynamic and allosteric protein both in its monomeric and filamentous forms. Further, alterations in

FIGURE 8. Uncorrected deuterium incorporation into specific peptides of yeast F (dark blue diamond), muscle F (pink square), sub1 F (brown triangle), and sub12 F-actin (green circle) with time. Peptides are represented in the monomer structure in Fig. 3. The number in parenthesis is the maximum number of exchangeable amide hydrogens. HD exchange data for peptides 145–65, 200–218, 206–218 and 286–299 are presented in supplemental Fig. 9. Subdomains are numbered 1–4.
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these allosteric interactions can produce biochemical differences that make different actin isoforms functionally suited for the roles they play in vivo. Our use of HD exchange has allowed us to delineate conformational changes without the complications that could arise from the introduction of large spectroscopic probes that might alter protein structure. A frustration with the technique is that the spectrum of peptides detected in a particular analysis can be quixotic, thus preventing us from carrying out comparisons we would like to make. Nevertheless, our use of HD exchange has allowed us to gain insight into the alterations in the protein that contribute to actin isoform differences in solution behavior and perhaps their resulting differences in functional specialization.

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