A Structural Basis for 14-3-3σ Functional Specificity*

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The 14-3-3 family of proteins includes seven isotypes in mammalian cells that play numerous diverse roles in intracellular signaling. Most 14-3-3 proteins form homodimers and mixed heterodimers between different isotypes, with overlapping roles in ligand binding. In contrast, one mammalian isoform, 14-3-3σ, expressed primarily in epithelial cells, appears to play a unique role in the cellular response to DNA damage and in human oncogenesis. The biological and structural basis for these 14-3-3σ-specific functions is unknown. We demonstrate that endogenous 14-3-3σ preferentially forms homodimers in cells. We have solved the x-ray crystal structure of 14-3-3σ bound to an optimal phosphopeptide ligand at 2.4 Å resolution. The structure reveals the presence of stabilizing ring-ring and salt bridge interactions unique to the 14-3-3σ homodimer structure and potentially destabilizing electrostatic interactions between subunits in 14-3-3σ-containing heterodimers, rationalizing preferential homodimerization of 14-3-3σ in vivo. The interaction of the phosphopeptide with 14-3-3 reveals a conserved mechanism for phospho-dependent ligand binding, implying that the phosphopeptide binding cleft is not the critical determinant of the unique biological properties of 14-3-3σ. Instead, the structure suggests a second ligand binding site involved in 14-3-3σ-specific ligand discrimination. We have confirmed this by site-directed mutagenesis of three σ-specific residues that uniquely define this site. Mutation of these residues to the alternative sequence that is absolutely conserved in all other 14-3-3 isoforms confers upon 14-3-3σ the ability to bind to Cdc25C, a ligand that is known to bind to other 14-3-3 proteins but not to σ.

14-3-3σ denotes a family of ~30-kDa dimeric proteins found in all eukaryotic cells (1–5). This family of highly conserved proteins consists of seven distinct gene products in human cells (β, γ, ε, η, σ, τ, and ζ) as well as a variety of post-translationally modified forms (6–9). To the extent that it has been investigated, broad heterodimerization has generally been observed between the different 14-3-3 isoforms (10, 11). In addition, all 14-3-3 proteins bind to common phosphoserine/phosphothreonine-containing peptide motifs corresponding to Mode-1 (RSXP) or Mode-2 (RXXXpXP) sequences (12).

14-3-3 proteins participate in a wide variety of signal transduction processes including Ras-Raf-mediated activation of the mitogen-activated protein kinase pathway, regulation of apoptosis, adhesion-dependent integrin signaling, and cell cycle control in response to genotoxic stress (1–5). All of these processes appear to involve multiple 14-3-3 isoforms. Although some isotype-specific differences in signaling clearly exist, the molecular basis for these differences remain obscure and difficult to rationalize given the strong sequence conservation and broad heterodimerization observed among the individual 14-3-3 proteins.

We have been particularly interested in understanding the roles of different 14-3-3 proteins in cell proliferation, cell cycle control, and human tumorigenesis (5, 13, 14). In epithelial cells, one particular 14-3-3 isoform, 14-3-3σ, appears to play a particularly important role in this regard. Hermeking et al. (15) demonstrated that 14-3-3σ was a major p53 response gene in HCT116 colon carcinoma cells following exposure to DNA damaging agents and suggested a role for 14-3-3σ in G2/M checkpoint function. Chan et al. (16) found that 14-3-3σ−/− HCT116 cells were unable to maintain the G2/M checkpoint arrest following exposure to adriamycin and subsequently died by mitotic catastrophe. One well established function of 14-3-3 in negatively regulating M-phase entry involves functional sequestration of the mitotic promoting phosphatase Cdc25C (17–20). Intriguingly, however, 14-3-3σ does not bind to Cdc25C (16, 21), suggesting that alternative σ-specific ligands, whose identities remain unknown, must also play important roles in controlling progression from G2 into M by some Cdc25C-independent mechanism.

Given the importance of 14-3-3σ in mitotic regulation, it is paradoxical that many different types of epithelial cancers have recently been found to down-regulate 14-3-3σ expression at the mRNA and protein level, either through promoter methylation and gene silencing (22–26) or through up-regulation of a specific E3 ubiquitin ligase that targets 14-3-3σ for destruction (27). This may be related to an alternative function of 14-3-3σ in promoting cell senescence, although to date this has only been demonstrated in human keratinocytes (28).

Why is 14-3-3σ apparently unique among the mammalian isoforms? What is structural basis for the distinct function of σ and its ligand discrimination? Why are 14-3-3σ effects not diluted out through heterodimerization with other 14-3-3 isoforms? This study was designed to investigate structural differences between 14-3-3σ and other 14-3-3 family members in an attempt to provide a molecular rationalization for some of these aspects of σ-specific function.

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FIG. 1. Sequence conservation among 14-3-3 proteins. A, sequence alignment. ClustalW-based sequence alignment of all human (h) 14-3-3 isotypes, the 14-3-3 protein from the budding yeast *Saccharomyces cerevisiae* (sc), and the fission yeast *Saccharomyces pombe* (sp). Inverted triangles demarcate residues at the dimerization interface; solid circles indicate a critical three-amino acid alteration in 14-3-3/H9268 involved in substrate discrimination. B, 14-3-3 cladogram. A rooted phylogenetic tree of the sequences in A generated using PHYLIP (40) reveals that 14-3-3σ is evolutionarily distinct from clades encompassing all other 14-3-3 isotypes.
MATERIALS AND METHODS

Construct Design and Recombinant Protein Expression—The pCDNA3.1 vector for mammalian expression of HA-14-3-3 was designed previously (21). The HA-tagged 14-3-3 was expressed using the HaTagGAGAAGCCACCATGATACAGATGACGACGATAAGG-3 vector. Where indicated, cells were treated with CS-112 (29) or with rabbit or goat polyclonal antibodies against 14-3-3 (panels a–c) or with rabbit polyclonal antibodies against the HA epitope (clone 12CA5; Roche Applied Science) or 14-3-3-3 (panels a–c). pTRE (BD Biosciences). Low level expression of FLAG-14-3-3 constructs (4) were obtained by culturing transfected cells in media lacking tetracycline. pGEX vectors for bacterial expression of glutathione S-transferase-tagged 14-3-3 were described previously (12). A pGEX vector for bacterial expression of glutathione S-transferase-14-3-3 fusion proteins was generated by PCR amplifying 14-3-3 from pGEX-14-3-3 with NcoI/HindIII compatible ends and subcloning into the HindIII/XhoI sites of the pCDNA3.1 + 2×FLAG vector. The final 2×FLAG-14-3-3 cassette was then subcloned into the BamHI/EcoRI sites of the tetracycline-inducible vector pTRE (BD Biosciences). Low level expression of 2×FLAG-14-3-3 was obtained by culturing transfected cells in media lacking tetracycline. 

pGEX vectors for bacterial expression of glutathione S-transferase-tagged 14-3-3 were described previously (12). A pGEX vector for bacterial expression of glutathione S-transferase-14-3-3 fusion proteins was generated by PCR amplifying 14-3-3 from pGEX-14-3-3 with NcoI/HindIII compatible ends and subcloning into the Neol/HindIII sites of the pCDNA3.1 + 2×FLAG vector. The final 2×FLAG-14-3-3 cassette was then subcloned into the BamHI/EcoRI sites of the tetracycline-inducible vector pTRE (BD Biosciences). Low level expression of 2×FLAG-14-3-3 was obtained by culturing transfected cells in media lacking tetracycline.

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Bacterial expression of recombinant proteins was performed by transforming BL21(DE3) E. coli with the appropriate plasmids, followed by growth in LB medium containing ampicillin. Cultures were induced at an A_{600} of 0.6–0.8 by addition of isopropyl β-D-thiogalactoside to a final concentration of 0.4 mM followed by growth for 4 h at 37 °C prior to cell harvesting by centrifugation.

Tissue Culture and Mammalian Cell Transfections—U2OS cells (passage 12, ATCC) were cultured in Dulbecco’s modified Eagle’s medium/HEPES medium supplemented with 10% fetal calf serum, glutamine, and penicillin/streptomycin in 10-cm2 culture dishes. The HA- and FLAG-tagged 14-3-3 constructs (4 μg) were transfected into HEK293 cells by the lipofection method with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Transfected cells were treated with 10 μg of insulin binding 24 h following transfection.

Preparation of Whole Cell Lysates, Immunoprecipitation, and Western Blot Analysis—Whole cell lysates were prepared by scraping U2OS cells into medium radioimmuno precipitation assay buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, containing 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM Na_{3}VO_{4}, 20 mM microcystin LR, 5 mM okadaic acid, 2 μM calpain, 0.0007% β-protomerotransiase, 10 μM E64, and 1 mM AEBPF) and pushed through a syringe equipped with a 30-gauge needle three times. Lysates were incubated for 15 min at 4 °C, centrifuged at 12,000 × g for 15 min at 4 °C, and the supernatants recovered. Protein concentration was measured using the Bio-Rad protein assay kit.

Immunoprecipitations were performed using 1 μg of whole cell lysates and 4 μg of the appropriate antibodies overnight at 4 °C. Following binding to protein-A-Sepharose beads for 1 h at 4 °C, the immunoprecipitates were washed three times with modified radioimmuno precipitation assay buffer, suspended in Laemmli sample buffer, resolved on SDS-PAGE gels, and transferred to polyvinylidene difluoride membranes. The membranes were blocked for 1 h at 4 °C using 5% nonfat dry milk (Bio-Rad) in phosphate-buffered saline containing 0.05% Tween 20, followed by incubation for 1–2 h at room temperature or overnight at 4 °C with mouse monoclonal antibodies specific for the HA epitope (clone 12CA5, Roche Applied Science) or 14-3-3 (clone CS-112 (29)) or with rabbit or goat polyclonal antibodies against 14-3-3 (C-20), e-Raf (C-12), and Cdc25C (C-19) or an antibody that recognizes all 14-3-3 isotypes (K-19), all from Santa Cruz Biotechnology (Santa Cruz, CA). Primary antibodies were used at dilutions between 1:1000 and 1:2500. Blots were washed three times for 15 min each with phosphate-buffered saline containing 0.05% Tween 20, followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibodies at 1:2500–1:5000 dilution for 60 min at room temperature. Following washing, immunoreactive bands were visualized by enhanced chemiluminescence (PerkinElmer Life Sciences) and the blots exposed to x-ray film (Eastman Kodak Co.).

Two-dimensional Gel Electrophoresis—For two-dimensional gel electrophoresis, immunoprecipitations from whole cell extracts were separated in 100 μl of focusing buffer (8% urea, 2% thiourea, 4% CHAPS, 15 mg/ml DTT, 2% ampholytes) cup-loaded onto prehydrated 7-cm IPG strips (pH 4–7, all strips from the same lot), and subjected to isoelectric focusing for 15 min at 100 V, 15 min at 200 V, 30 min at 500 V, 1 h at 1000 V, and 6 h at 3500 V using an IPGPhor isoelectric focusing system.

**FIG. 2.** 14-3-3-3 primarily forms homodimers in cells. **A**, homodimerization revealed by two-dimensional gel Western blot analysis. U2OS whole cell lysates were immunoprecipitated with antibodies against 14-3-3 (panels a–c) or 14-3-3 (panels d–f) and analyzed for co-associated 14-3-3 isotypes by two-dimensional gel electrophoresis and Western blotting. The two spots observed for 14-3-3 (a–c) indicates post-translational modifications. A fraction of 14-3-3 is known to be phosphorylated on Ser-185 (6); the post-translational modification of 14-3-3 is not known. B, antibody controls. Bacterial lysates containing recombinant glutathione S-transferase-14-3-3 fusion proteins were separated by SDS-PAGE and blotted with the antibodies used in A. A duplicate gel was stained with Coomassie Blue (lower panel). C, homodimerization confirmed by epitope tagging. U2OS cells were transfected with 2×FLAG-tagged 14-3-3. Total cell lysates (TCL) were prepared 24 h later, incubated with bead-immobilized anti-FLAG antibodies, and the immunoprecipitated proteins (IP) released under non-reducing conditions to minimize interfering signals from the antibody light chain. Under these conditions, the 2×FLAG-tagged 14-3-3 remained bound to the antibody-immobilized beads. Total cell lysate and immunoprecipitated protein samples, along with unbound material (Flow-thru) in the anti-FLAG-depleted lysates, were analyzed by blotting with the indicated antibodies.
system device (Amersham Biosciences). Strips were washed in re-equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS) in the presence of 20 mg/ml dithiothreitol for 10 min and then in the presence of 25 mg/ml iodoacetamide for 10 min to irreversibly modify all cysteine residues. The strips were then loaded onto 12% SDS-PAGE gels and electrophoresed at 120 V for 1 h. Gels were transferred onto polyvinylidene difluoride membranes and used for Western blot analyses using the appropriate antibodies in conjunction with enhanced chemiluminescence detection.

TABLE I
Crystallographic data and refinement statistics

| Space group: | C2221, \( a = 56.2 \, \text{Å}, b = 137.1 \, \text{Å}, c = 155.3 \, \text{Å} \) |
|-------------|--------------------------------------------------|
| Asymmetric unit: | 14-3-3 dimer with a phosphopeptide bound to each monomer |
| Data collection: | |
| Wavelength (Å): | 0.97949 |
| Resolution (Å): | 40–2.4 |
| \( R_{\text{sym}} \) \(^a\): | 0.095 (0.652) \(^b\) |
| Completeness (%): | 99.2 (99.3) |
| Unique \( hkl \) reflections: | 24,430 |
| Redundancy (≥fold): | 15.1 (13.5) |
| Average \( I/\sigma \): | 30.7 (5.4) |
| Refinement: | |
| \( hkl | I(h) > 0 \) reflections: | 22,712 |
| \( hkl \) (test set): | 2241 |
| \( R_{\text{work}} \) \(^{c}\): | 23.3 |
| \( R_{\text{free}} \) \(^{d}\): | 28.3 |
| Root mean square deviations: | |
| Bonds (Å): | 0.007 |
| Angles (degrees): | 1.07 |

\(^a\) \( R_{\text{sym}} = \sum_{h} I(h) - (I(h)) / \sum_{h} (I(h)) \), where \( I(h) \) is the \( h \)th reflection of index \( h \) and \( \langle I \rangle \) is the average intensity of all observations of \( I(h) \).

\(^b\) Values in parentheses are for the highest resolution bin (2.49–2.40 Å).

\(^c\) \( R_{\text{work}} = \sum_{h} |F_{\text{calc}}(h) - F_{\text{obs}}(h)| / \sum_{h} |F_{\text{obs}}(h)| \), calculated over the 90% of the data in the working set.

\(^d\) \( R_{\text{free}} \) is equivalent to \( R_{\text{work}} \) except calculated over the 10% of the data assigned to the test set.

RESULTS AND DISCUSSION

A comparison of the sequence of 14-3-3σ with that of other human 14-3-3 isotypes, as well as with those in the evolutionarily distant budding and fission yeast (Fig. 1A), reveals many regions of considerable sequence conservation. Despite this strong global sequence preservation, there are a number of short regions in 14-3-3σ that differ in general from those in other 14-3-3 proteins. A particularly striking example is a unique 3 amino acid substitution in the αH-α linker. Phylegetic analysis (Fig. 1B) reveals that σ is the most divergent of all 14-3-3 proteins, consistent with its apparently unique function in vivo. All other 14-3-3 proteins can be clustered into three major clades, one containing 14-3-3ε together with the yeast isoforms, a second clade containing the γ and η isoforms, and a third clade containing the τ, β, and ζ isoforms. The structure of 14-3-3σ gene on chromosome 1, together with its complete lack of introns (a feature unique to σ), support a hypothesis that 14-3-3σ may represent a functional retrotransposed gene.

Given the propensity of 14-3-3 proteins to form both homodimers and mixed heterodimers with other 14-3-3 isotypes (10, 11), what accounts for the functional specificity of 14-3-3σ revealed by mammalian genetic experiments (16)? To address this question, the extent of 14-3-3σ homo- versus heterodimerization was investigated by immunoprecipitation of endogenous 14-3-3σ in U2OS cells and the results compared with those obtained by immunoprecipitating 14-3-3β. Samples were analyzed by two-dimensional gel electrophoresis and Western blotting to maximize resolution between individual isoforms. As shown in Fig. 2A, 14-3-3β was easily detectable in anti-β immunoprecipitates (panel a); however, no 14-3-3σ was pulled down in these samples (panel b). In contrast, a monoclonal antibody against 14-3-3σ strongly immunoprecipitated 14-3-3σ (panel c) but failed to immunoprecipitate any 14-3-3β (panel d). These data suggest that endogenous 14-3-3σ and β do not form mixed heterodimers. When the anti-β immunoprecipitates were blotted with an antibody that recognizes all of the 14-3-3 isotypes, at least 10 distinct spots were observed (panel e), consistent with extensive heterodimerization between β and
other 14-3-3 isoforms. In contrast, when the anti-σ immunoprecipitates were probed with this same antibody (panel f), there were very few additional spots seen. These data indicate that 14-3-3σ preferentially forms homodimers within cells.

The specificity of the antibodies used in Fig. 2A is shown in Fig. 2B. To verify that the lack of 14-3-3σ heterodimerization we observed was not an artifact of dimer disruption by the anti-σ antibody, we transfected cells with a 2×FLAG-tagged 14-3-3σ construct and looked at its co-association with endogenous σ and β in anti-FLAG immunoprecipitates. As shown in Fig. 2C, endogenous 14-3-3σ dimerized with the 2×FLAG-tagged σ, while endogenous 14-3-3β did not. These findings confirm the conclusion, drawn from our two-dimensional gel results, that 14-3-3σ preferentially forms homodimers.

To understand the basis of preferential homodimerization, and to shed light on mechanisms of functional specificity for 14-3-3σ, we solved the x-ray crystal structure of 14-3-3σ bound to the mode-1 phosphopeptide MARSHpSYPAKK at 2.4 Å resolution (Table I). As shown in Fig. 3A, the general shape of 14-3-3σ is essentially identical to that of 14-3-3γ and -ζ (34, 35), consisting of nine antiparallel α-helices forming a horseshoe-shaped dimer. The basis for dimer formation in 14-3-3σ is essentially the same as that of these other isoforms and involves a series of distributed interactions from residues in helices αA and αB in one monomer with residues in helices αC and αD′ in the other (Figs. 1A and 3B).

For simplicity, we focus further comparisons of the 14-3-3σ structure to that of 14-3-3γ bound to the same mode-1 phosphopeptide (12), although the conclusions apply equally to those obtained by comparing 14-3-3σ with the unliganded...
In 14-3-3/H9270 the angle between A and B is different from that in the dimer. This difference may be related to the unique sequence of the BC loop in 14-3-3/H9268 compared with that in other human isoforms. Across the dimer interface in the opposing monomer, the loop connecting helices C and D, which contains two extra amino acids in 14-3-3/H9268, is disordered and helix D is one turn shorter at its amino-terminal end than in the 14-3-3/H9256 structure. Helices A and D in the 14-3-3σ structure are observed to pack slightly closer together across the interface than in C (Fig. 3C), perhaps as a consequence of these changes.

There are seven amino acid differences between 14-3-3/H9268 and 14-3-3/H9256 at the dimer interface (six between σ and τ). Five of these amino acid substitutions are σ-specific and must provide a structural basis for σ homodimerization through stabilizing homodimeric interactions and destabilizing heterodimeric in-
tections. In the 14-3-3-3 structure, Phe-25, which is unique to σ, makes a ring-ring interaction across the interface with Tyr-84 on αD (Fig. 3D). The aromatic ring of the Phe-25 side chain prevents Lys-9 (on αA) from adopting the conformation it has in the 14-3-3C structure. Instead, in the 14-3-3σ structure Lys-9 participates in a salt bridge with Glu-83 located at the NH2-terminal end of αD. Because of 2-fold symmetry, these σ-specific ring-ring and salt-bridge interactions occur twice in the dimer. Thus, the 14-3-3σ homodimer has four stabilizing interactions that cannot occur in heterodimers that include 14-3-3A.

Furthermore, Ser-5 and Glu-80, both unique to 14-3-3σ (Fig. 1A), lie opposite each other at the dimer interface (Fig. 3B), near the disordered NH2 terminus of αD of 14-3-3σ. In 14-3-3σ-containing heterodimers with the τ, ζ, β, or ε isoforms, Glu-80 in σ would be in close proximity to the Asp or Glu side chain that replaces Ser-5 of σ (Fig. 3E), resulting in a highly unfavorable interaction between these two opposing negative charges. Thus, in addition to the loss of the two stabilizing homodimeric interactions mentioned above, heterodimers of 14-3-3σ with these isoforms would be required to tolerate an additional highly destabilizing interaction across the dimer interface. Furthermore, favorable packing of the bulky hydrophobic Met or Leu sidechains corresponding to Glu-80 in the ε, β, ζ, τ, and γ isoforms may be incompatible with the subtle shifts in the positions of αA and αB in 14-3-3σ. Taken together, these observations suggest a structural basis for our results showing that the σ isoform preferentially forms homodimers.

Each of the C2 symmetry related monomers of the 14-3-3σ dimer contains a phosphopeptide binding pocket running in opposite directions, in which residues 5–10 of the peptide (Ser-His-Ser(P)-Tyr-Pro-Ala) are clearly visible. On one side, poor but interpretable density is present for residues 2–4 (Gly-Ala-Arg). The conformation of the bound phosphopeptide is essentially the same as that in the corresponding complex with 14-3-3C. For each monomer, the peptide binds in a narrow cleft formed primarily by the surface-exposed residues from αA, αB, αC, and αD on the concave surface of the molecule (Fig. 4A). The topography of the cleft requires that the peptide have a distinct kink that allows it to exit the channel after which it becomes disordered in the structure. This kink is produced by a cis-Pro residue in the +2 position. The details of the interaction between the protein and the phosphopeptide are the same as in the 14-3-3σ-mode1 peptide complex. All of the key interactions, including those involved in coordinating the phosphate group of the phosphoserine (Fig. 4B), are essentially identical. This is not surprising, as the sequence identity of the residues that comprise the phosphopeptide-binding site are strictly conserved (Fig. 4C). Consequently, the selectivity of 14-3-3σ selectivity for specific ligands must involve other sites besides the major phosphopeptide-binding groove.

Most of the non-conserved residues are exposed on the opposite face of the dimer from the concave depression that contains the phosphopeptide binding site. However, a patch of three residues in the αH-1 linker (Met-202, Asp-204, and His-206), strictly conserved in every sequence in Fig. 1A except 14-3-3σ, is exposed at the extreme ends of the ridges at the tops of either side of the U-shaped dimer. These three residues form a small island of exposed non-conserved sequence surrounded mostly by conserved sequence (Fig. 4C). This observation suggests that this site might constitute a 14-3-3σ-specific binding site for other protein ligands. Intriguingly, the patch formed by these three residues lies adjacent to the invariant Phe-198 (position 204 in the alignment in Fig. 1A), whose mutation in the Drosophila epsilon isoform appears to impair Raf/14-3-3 interactions required for Ras-mediated signaling (36). In addition, the αH-1 linker in 14-3-3C makes direct contact with part of serotinin N-acetyltransferase in the 14-3-3C-AANAT co-crystal structure (37). These findings further suggest that this region of 14-3-3σ may indeed be part of a second ligand binding surface.

To investigate this, and determine whether this site was involved in σ-specific ligand discrimination, we mutated Met-202, Asp-204, and His-206 in σ to the Ile, Glu, and Asp residues that are present in all other non-σ isoforms and transfected the resulting HA-tagged construct into U2OS cells (Fig. 4D). Both wild-type and the triple mutant σ bound to c-Raf-1 in cells. In contrast, wild-type σ did not bind significantly to Cdc25C, whereas the triple mutant form of σ showed robust binding, as well as a slight increase in Cdc25C binding after irradiation-induced DNA damage, particularly when normalized to the amount of HA-14-3-3σ in the IPs. These findings strongly suggest that this region in 14-3-3σ normally constitutes a second phospho-independent ligand-binding site that is likely to be involved in mediating selection for and against specific ligands. Similar phospho-independent ligand-binding surfaces that are spatially separate from the phosphopeptide-binding pocket have also been observed in other phosphoserine/threonine-binding domains (38).

In conclusion, we have shown that endogenous 14-3-3σ preferentially forms homodimers in cells, a finding that can be rationalized at the structural level by sequence differences and selective interactions at the dimer interface. The structural basis of 14-3-3σ phosphopeptide-binding is essentially identical to that seen in 14-3-3C phosphopeptide and protein complexes (12, 37, 39), suggesting that 14-3-3σ-specific substrate discrimination involves alternative secondary surfaces. We have identified one of these surfaces in the αH-1 linker involving residues Met-202, Asp-204, and His-206. Mutation of this α-specific surface patch to the corresponding sequence in other 14-3-3 isoforms causes 14-3-3σ to bind to Cdc25C, a molecule that normally binds to other 14-3-3 isoforms but not to σ. These findings may assist in understanding the molecular basis for 14-3-3σ-specific function in cell cycle control and cancer.

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X-ray Crystal Structure of 14-3-3σ

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