The Jak1 SH2 Domain Does Not Fulfill a Classical SH2 Function in Jak/STAT Signaling but Plays a Structural Role for Receptor Interaction and Up-regulation of Receptor Surface Expression*§

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The presence of a Src homology 2 (SH2) domain sequence similarity in the sequence of Janus kinases (Jaks) has been discussed since the first descriptions of these enzymes. We performed an in depth study to determine the function of the Jak1 SH2 domain. We investigated the functionality of the Jak1 SH2 domain by stably reconstituting Jak1-defective human fibrosarcoma cells U4C with endogenous amounts of Jak1 in which the crucial arginine residue Arg466 within the SH2 domain has been replaced by lysine. This mutant still binds to the receptor subunits gp130 and OSMR. Moreover, the SH2 R466K mutation does not affect the subcellular distribution of Jak1 as assessed by cell fractionation and confocal microscopy of cells expressing endogenous levels of non-tagged or a yellow fluorescent protein (YFP)-tagged Jak1-R466K, respectively. Likewise, the signaling capacity of Jak1 was not affected by this point mutation. However, we found that the SH2 domain is structurally important for cytokine receptor binding and surface expression of the OSMR.

The Janus family of protein-tyrosine kinases comprises four mammalian members. Three, Jak1, Jak2, and Tyk2, are expressed in a wide variety of tissues, whereas Jak3 expression is restricted to cells of the hematopoietic system. Jak1 is membrane-localized by binding to cytokine receptors (1). It is involved in signal transduction of several cytokines including interferons (IFNα, IFNβ, and IFNγ) as well as interleukin-6 (IL-6)-type cytokines, OSM (oncostatin M), interleukin-11 (IL-11), leukemia inhibitory factor, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC)). IL-6-type cytokines signal either via homodimers of the signal transducing receptor subunit gp130 or via heterodimeric receptor complexes containing gp130 together with the leukemia inhibitory factor receptor (LIFR) or the OSMR (for a review, see Ref. 2). All these signal transducing receptor subunits have been described to bind Jak1, Jak2 and Tyk2. Among them, Jak1 is essential for signal transduction as demonstrated for Jak1-deficient fibrosarcoma cells and for cells derived from Jak1 knock-out mice (3, 4). Interestingly, the surface expression of the OSMR and other receptors (5–7) have been described to be dependent on Jak binding.

The molecular mechanism of Jak activation upon cytokine stimulation is not understood. It is still under debate which functional domains exist in the Jaks, and the interplay of these domains in kinase activation is not clear. Based on sequence similarities between the Jaks (molecular masses of 120–140 kDa), seven Jak homology (JH) regions have been defined (Fig. 2A) that match the more recently predicted domain structure only partially (8). The JH1 domain, a classical tyrosine kinase domain, is flanked by a non-functional kinase domain, the pseudokinase domain (JH2) that may play a regulatory role (9, 10). The N-terminal half of the Jaks, domains JH3 to JH7, contains a predicted FERM domain (8) and a putative SH2 domain. The FERM domain is involved in binding to cytokine receptors (11, 12) and fixes the Jak permanently to the receptor, resulting in a complex that can be compared with a receptor tyrosine kinase (1, 13, 14). The presence of an SH2 domain sequence similarity (C-terminally to the FERM domain) has been discussed since the first description of Jaks (15, 16) and with the improvement of structure prediction tools the number of studies finding SH2 domain sequence similarities in Jaks have increased (17–19).

SH2 domains contain about 100 amino acids and consist of two α-helices and a central antiparallel β-sheet. They bind to specific motifs containing a tyrosine residue. Phosphorylation of this tyrosine is a prerequisite for SH2 domain binding. SH2 domains contain a conserved arginine at the β5 position, which contacts the phosphotyrosine in the motif recognized by the SH2 domain and makes a crucial contribution to the affinity. Non-functional SH2 domains can be generated by mutation of the crucial arginine residue to lysine (20–23).

In the present study we investigate the effects of an inactivating mutation of the Jak1 SH2 domain, Jak1-R466K, in stable transfectants. Whereas we did not find an effect of this mutant on IL-6-type cytokine or IFNγ/IFNα signaling, deletion

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The abbreviations used are: Jak, Janus kinase; FERM, four-point-one, ezrin, radixin, moesin; GFP, green fluorescent protein; IFN, interferon; IL, interleukin; JH, Jak homology; OSM, oncostatin M; R, receptor; SH2, Src homology 2; STAT, signal transducer and activator of transcription; Tyk, tyrosine kinase; YFP, yellow fluorescent protein; MHC-I, major histocompatibility complex, class I; HA, hemagglutinin; FACS, fluorescence-activated cell sorter; PBS, phosphate-buffered saline.
mutants revealed that the SH2 domain is of structural importance for the previously described regulation of OSMR surface expression by Jak1.

**EXPERIMENTAL PROCEDURES**

**Structural Alignment of SH2 Domains—**The initial alignment of the SH2 domain sequences of Jak1, Jak2, Jak3, and Tyk2 from different species (h, human; m, mouse; r, rat; c, chicken; p, pig; q, rhesus macaque; z, zebrafish; y, carp and t, puffer fish) and of the Brca2/melanogaster Jak3 kinase homologue Hop with the sequences of the structurally explored SH2 domains of the human c-Src protein-tyrosine kinase, the human phosphatidylinositol 3-kinase p85 subunit, the bovine phospholipase C-γ, the human Bcr-abl protein-tyrosine kinase, and the N-terminal and C-terminal SH2 domains of human SHP-2 protein-tyrosine phosphatase was performed by the use of the BLAST program (24). Modifications were introduced to meet structural requirements derived from the known SH2 structures. The sequential alignment of the known structures is based on the direct superposition of their backbone coordinates. Brookhaven data bank entry codes for the used structures are: 1hcs, 1a1b, and 1shd for human c-Src (25–27); 1pic for human phosphatidylinositol 3-kinase p85 (28); 2pid for bovine phospholipase C-γ1 (29); 2abl for human Bcr-abl (30); and 2SHP for human SHP-2. ROSETTA-Prot/TEMBL accession numbers for the sequences used are: P23458 (hJak1), P52332 (mJak1), Q8PWM9 (cJak1), Q10290 (zJak1), O57612 (tJak1), Q97TJ1 (pJak1), Q9NN47 (qJak1), O35803 (rJak1), Q09178 (yJak1), Q62689 (rJak2), Q75R65 (cJak2), Q9PFD2 (tJak2), Q62120 (mJak2), Q06674 (hJak2), O19064 (pJak2), Q95396 (zJak2), P29597 (tTyk2), Q9PFD1 (yTyk2), Q8R117 (mTyk2), P52333 (rJak3), Q0B8Y2 (mJak3), Q9PFD0 (hJak3), Q6PTN6 (yJak3), Q8DPC5 (cJak3), Q52243 (zJak3). For the backbone coordinate alignment of the SH2 domain sequences excluding the Janus kinases an initial alignment was performed with the MULTALIN (v5.4.1) software (32) using the Src domain sequences excluding the Janus kinases and the respective antibodies. Anti-Jak1 (HR785, Santa Cruz Biotechnology) or anti-IL5R (enhanced yellow fluorescent protein) contained excitation at 514 nm, a dichroic mirror at 535 nm, and emission at 543 nm. The laser was a 488 nm argon laser. The LSM 510 is equipped with an HeNe laser (543 nm) (Zeiss, Jena, Germany). The laser was used for excitation of labeled samples. A confocal microscope was used to perform confocal imaging in living cells. The SWIFT is equipped with a 488 nm argon laser. The LSM 510 is equipped with an HeNe laser (543 nm) (Zeiss, Jena, Germany). The laser was used for excitation of labeled samples. A confocal microscope was used to perform confocal imaging in living cells.

**Cell Lysis, Immunoprecipitation, and Western Blot Analysis—**All steps of cell lysis and immunoprecipitation were performed at 4 °C using ice-cold buffers. Cytosolic, membrane, and nuclear fractions were prepared using the protocol described in detail before (1).

**Flow Cytometry—**Cells were resuspended in cold PBS supplemented with 5% fetal calf serum and 0.1% sodium azide (PBS/azide), 5 × 10^5 to 1 × 10^6 cells in 100 μl of PBS/azide were incubated with 1 μg/ml monoclonal anti-Neu (W6/32, Sigma), anti-OSMR (AN12, Santa Cruz Biotechnology), anti-STAT1 (S21120 from Transduction Laboratories), anti-phosphotyrosine (Pyr9, Santa Cruz Biotechnology), anti-STAT3 (S5359, Santa Cruz Biotechnology), anti-IL5R (enhanced yellow fluorescent protein) (9131 from Cell signaling), anti-phospho-STAT3 (Tyr705) (9171 from Cell signaling) and anti-STAT5 (Tyr541) (9171 from Cell signaling) were used for detection. The horseradish peroxidase-conjugated secondary antibodies were purchased from Dako. Signals were detected using the ECL system (Amersham Biosciences).

**FACS**—Cells were resuspended in cold PBS supplemented with 5% fetal calf serum and 0.1% sodium azide (PBS/azide), 5 × 10^5 to 1 × 10^6 cells in 100 μl of PBS/azide were incubated with 1 μg/ml monoclonal anti-Neu (W6/32, Sigma), anti-OSMR (AN12, Santa Cruz Biotechnology), anti-STAT1 (S21120 from Transduction Laboratories), anti-phosphotyrosine (Pyr9, Santa Cruz Biotechnology), anti-STAT3 (S5359, Santa Cruz Biotechnology), anti-IL5R (enhanced yellow fluorescent protein) (9131 from Cell signaling), anti-phospho-STAT3 (Tyr705) (9171 from Cell signaling) and anti-STAT5 (Tyr541) (9171 from Cell signaling) were used for detection. The horseradish peroxidase-conjugated secondary antibodies were purchased from Dako. Signals were detected using the ECL system (Amersham Biosciences).
FIG. 1. SH2 domain alignment of Jak1, Jak2, Jak3, and Tyk2 with other SH2 domains. The sequences of the Janus kinase SH2 domains of different origin (h, human; m, mouse; r, rat; c, chicken; p, pig; q, macaque; z, zebrafish; y, carp; and t, puffer fish) were aligned with the SH2 domain of human phosphatidylinositol 3-kinase p85 subunit (P85cC) (28), the bovine phospholipase C-γ (29), the human Bcr-abl protein-tyrosine kinase.
RESULTS

The Members of the Janus Kinase Family Contain a Divergent SH2 Domain—To determine whether the potential SH2 domain of Janus kinases possesses the features required for proper recognition of phosphotyrosine motifs, we performed a structural alignment of 25 SH2 sequences of Janus kinases with the sequences of structurally explored SH2 domains of other proteins (Fig. 1). To better evaluate the variability of the different amino acid positions, an additional alignment of 420 SH2 domain sequences excluding the Janus kinases was generated (data not shown). Residues conserved in SH2 domains to at least 30 or 90% are indicated in Fig. 1 in blue and red, respectively. We found that critical residues involved in the hydrophobic core of the domain (e.g. positions /H9251A9, /H9252B2, /H9252B3, /H9252B4, /H9251C3, /H9252C5, /H9252D7, /H9251B2, and /H9251B5) are strictly conserved in the 420 SH2 domains (hydrophobic amino acid side chains in 100% of the sequences) as well as in all the Jaks (highlighted by an h in Fig. 1). Furthermore, secondary structure prediction analysis of the Janus kinase family members revealed the typical secondary structure pattern found in SH2 domains (data not shown). For functionality, SH2 domains depend on the arginine residue at position /H9252B5, which contacts the phosphate group of a binding phosphotyrosine motif. Accordingly, this residue was present in 419 sequences (99.8%) in our alignment of 420 reference SH2 domain sequences, highlighting the strict requirement for this amino acid at this position. Strikingly, this arginine is not equally well conserved in all the Jaks. Whereas this residue is present in all the sequences of Jak1 and Jak3, it is exchanged to glutamine in one Jak2 sequence (puffer fish) and none of the Tyk2 sequences contains an arginine at this position (histidine in humans, glutamine in mouse, and cysteine in puffer fish). Thus, considering all the Jaks, there is a striking discrepancy of conservation between structural (conserved in all) and functional residues (conserved in only some Jaks). While this finding precludes a general requirement of the SH2 domain in Janus kinases, the fact that the crucial arginine is conserved in all Jak1 sequences may suggest that the SH2 domain may play a role in Jak1-mediated signal transduction. We therefore decided to generate a non-functional SH2 mutant of Jak1.

Mutation of Arg466 to Lysine Has No Effect on Jak1/Cytokine-Receptor Binding and Up-regulation of OSMR Surface Expression—To investigate the effects of a non-functional SH2 domain within Jak1, we introduced an arginine to lysine mutation at position /H9252B5 (amino acid 466) of Jak1 (Fig. 2A). First, we checked whether the mutation of this residue would affect Jak1/receptor binding. Due to low endogenous protein levels and lack of sufficiently efficient antibodies against gp130 or kinase (Abl) (30), as well as with the N- and C-terminal SH2 domains of murine SHP2 protein-tyrosine phosphatase (SHP2N, SHP2C) (31). Secondary structure characteristics are given on top following the common nomenclature (38). Residues that are highly conserved among the Janus kinase sequences are highlighted in green. Blue and red characters indicate residues conserved in SH2 domains to at least 30 or 90%, respectively (based on our alignment of 420 SH2 domain sequences). Amino acid positions critical for the build-up of the hydrophobic core of the domain where a hydrophobic amino acid side chain is present in 100% of the sequences are indicated by h. The arginine residue at position βB5 (•) that is crucial for the function of SH2 domains is highlighted in yellow.
OSMR the Jak/receptor binding was investigated by transiently expressing Jak1 constructs and a chimeric IL-5Rα/gp130-construct in COS-7 cells as described before (11). To monitor Jak association, the chimeric receptors were immunoprecipitated with an IL-5Rα-specific antibody and subsequently analyzed by Western blotting (Fig. 2B). Coprecipitated Jak1 was detected using a Jak1-specific antibody. As described previously (11), the IL-5Rα/gp130 constructs efficiently bound wild-type Jak1, whereas association of the non-binding mutant Jak1-L80A/Y81A was greatly impaired. The single amino acid exchange of the phosphotyrosine-binding residue in the Jak1 SH2 domain arginine 466 to lysine did not affect Jak association to the gp130 cytoplasmic part (Fig. 2B). The same results were obtained using an IL-5Rα/OSMR-construct (data not shown).

Since the Jak1 N terminus comprising the FERM and the SH2 domains is involved in cytokine receptor binding, and since Jak association to the OSMR has recently been shown to promote an up-regulation of the surface expression of this receptor (6), we hypothesized that the SH2 domain might play a role in the latter process. Upon coexpression of a GFP-tagged OSMR with different Jak mutants in COS-7 cells, both Jak1 and Jak1-R466K efficiently up-regulated the OSM receptor, while Jak1-L80A/Y81A, the non-receptor-binding mutant, failed to do so (Fig. 2C). Thus, the non-functional Jak1 SH2 domain mutant, Jak1-R466K, does not only have the ability to bind to cytokine receptors like wild-type Jak1 but also retains the ability to promote surface expression of the OSMR.

**Mutation of Arginine 466 to Lysine Has No Effect on the Subcellular Distribution of Jak1—**Jak membrane localization was recently shown to be dependent on cytokine receptor binding (1). To examine whether a low level constitutive phosphotyrosine/SH2 domain interaction could have an effect on Jak localization, we investigated the localization of Jak1, Jak1-R466K, and Jak1-L80A/Y81A in stably transfected U4C cells by using cell fractionation techniques (Fig. 3A). Jak1-L80A/Y81A was used as control, since it is known to be localized to the cytoplasm (1). The cells were fractionated and Jak1 was detected by Western blot analysis. The blots were counterstained with antibodies against marker proteins of the different subcellular fractions. Calnexin was used as membrane fraction marker and lamins A and C as nuclear marker proteins. Cyt., cytoplasmic; Men., membrane; Nuc., nuclear. **B**, the localization of the YFP-tagged proteins in living U4C cells stably expressing Jak1-YFP or the mutant Jak1-R466K-YFP was monitored by confocal microscopy.

**Mutation of Arginine 466 to Lysine Does Not Affect Signal Transduction through the Jak1/STAT Pathway—**We next compared the signal transducing capacity of Jak1 and Jak1-R466K in the stably reconstituted JAK1-deficient U4C cells. Cells expressing a kinase-negative Jak1 mutant Jak1-K907E were used as negative control. The cells were stimulated with different cytokines signaling via Jak1 (IL-6, OSM, IFNγ, and IFNα) and total cellular lysates were prepared. Part of each lysate was used for an immunoprecipitation with a Jak1 antibody. The immunoprecipitates and the lysates were then subjected to SDS-PAGE and Western blotting (Fig. 4A). Jak1-R466K does not show any differences in signal transduction compared with Jak1. Jak1 phosphorylation as well as STAT1 and STAT3 tyrosine phosphorylation after cytokine stimulation were identical in U4C-Jak1 and U4C-Jak1-R466K cells, whereas these signaling events were impaired in U4C-Jak1-K907E cells. Single clones and pools of stable U4C transfectants always showed identical results (data not shown).

To examine possible changes in STAT activation kinetics, the U4C-Jak1, U4C-Jak1-R466K, and U4C-Jak1-K907E cells were stimulated with OSM or IFNγ for different times, and total cellular lysates were prepared and analyzed by Western blotting (Fig. 4B). The extent and the kinetics of tyrosine phosphorylation of STAT1 and STAT3 after cytokine stimulation were identical in U4C-Jak1 and U4C-Jak1-R466K cells, whereas the negative control U4C-Jak1-K907E cells show disturbed signaling.

To monitor MHC-I gene expression upon IFNα or IFNγ stimulation, U4C-Jak1 and U4C-Jak1-R466K cells were stimulated for 3 days and then analyzed using flow cytometry (Fig. 4C). The up-regulation of MHC-I surface expression in U4C-Jak1-R466K in response to both cytokines was identical to the one found in U4C-Jak1 cells. U4C-Jak1-K907E control cells did not show up-regulation of MHC surface expression (data not shown).
FIG. 4. Mutation of Arg466 to lysine does not affect signal transduction through the Jak/STAT pathway upon cytokine stimulation.  

**A**, stably transfected U4C-Jak1, U4C-Jak1-R466K, and U4C-Jak1-K907E (kinase-dead mutant) cells were stimulated with IL-6, OSM, IFNγ, or IFNα and total cellular lysates were prepared. Part of the lysates was subjected to an immunoprecipitation with a Jak1 antibody. Lysates and immunoprecipitates were resolved by SDS-PAGE and transferred to membranes by Western blot. The blot of the immunoprecipitates was detected using a phosphotyrosine antibody and reprobed using a Jak1 antibody. The lysate blots were detected with phosphospecific STAT1 and STAT3 antibodies and reprobed with STAT antibodies. 

**B**, U4C-Jak1, U4C-Jak1-R466K, and U4C-Jak1-K907E cells were stimulated with OSM or IFNγ for different periods of time and total cellular lysates were prepared. Lysates were resolved by SDS-PAGE and transferred to membranes by Western blot. The blots were detected with phosphospecific STAT1 and STAT3 antibodies and reprobed with STAT antibodies. 

**C**, U4C cells stably expressing wild-type Jak1 or Jak1-R466K were stimulated for 3 days with IFNγ or IFNα, and MHC-I expression was monitored by FACS analysis using an MHC-I-specific antibody. Histograms from unstimulated cells are shown in gray, and those from cells treated with IFNα or IFNγ are depicted as solid or broken lines, respectively.
The SH2 Domain of Jak1 Is Structurally Important for Supporting OSMR Binding and Surface Expression—We generated a number of Jak1 constructs to test whether the SH2 domain is structurally needed for proper Jak1 binding to the OSMR and consequent receptor surface expression. All Jak1 constructs used in this assay are C-terminally tagged with GFP so that their overall expression can be easily measured by Western blot or FACS analysis (Fig. 5A). The construct Jak1-SH2Δ comprises the N terminus of Jak1 and has intact FERM and SH2 domains (residues 1–558). The construct JH4α has an intact FERM domain but only part of the SH2 domain (residues 1–456). This deletion disrupts the structural integrity of the SH2 domain. The FERMα construct represents the isolated FERM domain (residues 1–440). To test whether SH2 domains of other Jaks could substitute for the Jak1 SH2 domain, we generated constructs where the SH2 domains of Jak2 or Jak3 were fused to the FERMα construct (Fig. 5A, J2-SH2-GFP and J3-SH2 GFP).

We next studied the binding of the different Jak1 constructs to an IL-5Rβ/OSMRΔ1 chimera, which we previously used to monitor -Jak1 receptor association (Fig. 5B) (6). The chimeric receptor was precipitated with an IL-5Rβ antibody and coprecipitated Jak1 was detected with an antibody recognizing the GFP moiety of wild-type Jak1 and the Jak mutants. As can be seen in Fig. 5B (left panels), wild-type Jak1 and Jak1-SH2Δ can be efficiently precipitated with the chimeric OSMR, whereas the shorter Jak1 proteins Jak1-JH4α and Jak1-FERMα show a decreased binding. We also found that the construct containing the Jak2 SH2 domain fused to the Jak1 FERM domain (J2-SH2) efficiently bound to the IL-5Rβ/OSMR, whereas the fusion construct encompassing the Jak3 SH2 domain (J3-SH2) did not bind (Fig. 5B, right panels). Similarly, a chimera in which not only the SH2 domain of Jak3 but the whole C terminus of Jak3 is present is also deficient in receptor up-regulation (supplemental Fig. 1). This band has an intact FERM domain. The FERMα construct is lost if these residues are mutated (SH2Δ-L80A/Y81A). We therefore introduced an arginine to lysine point mutation (R466K) into Jak1, which impairs the function of the SH2 domain but does not interfere with the structural integrity of the domain. This exchange is a common loss of function mutation used in SH2 domain studies (20–23). It is commonly accepted that the lesser length of a lysine causes a disruption of the binding to the phosphate oxygens of the phosphotyrosine residue (20). In addition, the positively charged amino group from the lysine cannot mimic the binding of the terminal guanidinium nitrogen of the arginine to the phosphate oxygens.

We show that mutation of R466 to lysine has no effect on Jak1/ cytokine-receptor binding as well as on its subcellular distribution; subcellular fractionation experiments and confocal microscopy with YFP-tagged Jak1 and Jak1-R466K showed that the mutant is localized to membranes as is the wild-type. Thus, there does not seem to be any basal phosphotyrosine/SH2 interaction involved in the localization of Jak1.

Next, we investigated the effect of the Jak1 SH2 domain knock-out R466K on signal transduction in the context of different cytokine receptor complexes. The IL-6- and OSM-receptor complexes represent a setting of cytokine receptors using predominantly Jak1 (3, 4) but can promiscuously recruit Jak2 and Tyk2. In the IFNγ receptor, the Jak1 SH2 knock-out mutant is paired with a Jak2 containing an intact SH2 domain. Finally, in the case of the IFNα receptor complex, the Jak1 SH2 mutant is paired with Tyk2, which is naturally defective in SH2 function (Fig. 1). STAT factor activation kinetics and Jak1 activation itself was unchanged in stable Jak1-R466K transfectants upon stimulation with any of these cytokines. Since even IFNα stimulation in U4C-Jak1-R466K cells shows unaltered Jak and STAT activation as well as an efficient up-regulation of MHC-1 in comparison to Jak1-WT, it is clear that there is no need for any functional Jak SH2 domain in IFNα signaling. Thus, we conclude that in case of the tested cytokines, the SH2 domain of Jak1 does not contribute to signaling via the Jak/STAT pathway. As in Jak1, the crucial arginine is conserved in all available Jak3 sequences. Interestingly, it was reported recently that this arginine is mutated to histidine in a SCID patient. Ectopic expression of this mutant in HeLa cells revealed an altered subcellular localization (37). It is an intriguing thought that a functional SH2 domain may be required for Jak3 signaling.

We demonstrated in a previous study that Jak1 is important for efficient surface expression of the OSMR. In particular, we could show that this effect is mediated by binding of Jak1 to the receptor but that Jak1 kinase activity is not required, since a kinase-negative mutant (K907E of Jak1) was as efficient as wild type Jak1 in mediating OSMR surface expression. Further data suggested that the OSMR contains a negative regulatory signal in its membrane proximal region that may be masked by Jak1 upon its binding to the receptor (6).

Here we show that a truncated Jak1 encompassing its N-terminal region can bind to the OSMR and up-regulate its surface expression as efficiently as full-length Jak1 demonstrating that the C-terminal part of the enzyme does not structurally contribute to receptor association and up-regulation. However, we found the SH2 domain of Jak1 to be struc-
FIG. 5. The SH2 domain of Jak1 is structurally important for supporting OSMR binding and surface expression. A, schematic representation of the Jak1 constructs used to study the binding of Jak1 to the OSMR. B, COS-7 cells were transfected with the chimeric receptor IL-5Rβ/OSMRΔ1 together with the indicated GFP-tagged Jak1 constructs in a pSVL vector. Cells were stained with antibodies against IL-5Rβ and secondary antibody. The surface expression of chimeric receptors in cells displaying similar GFP-fluorescence was analyzed by FACS analysis. The values obtained for cells expressing the non-binding Jak1 mutant L80A/Y81A were set to 100%. Mean values and standard deviation obtained from at least four independent experiments are depicted (bar diagram). For Western blot analysis, cells were lysed, and IL-5Rβ/OSMRΔ1 was precipitated using an antibody recognizing IL-5Rβ. Using a GFP antibody, coprecipitation of the GFP-tagged Jak constructs and expression levels in whole cell lysates were monitored. C, Jak1-deficient U4C cells were transfected with Jak1-GFP constructs (in a pcDNA3 vector) or with empty vector. Surface expression of the endogenous OSMR was monitored by FACS analysis using a monoclonal antibody against the OSMR. The values obtained for mock-transfected cells were set to 100%. Mean values and standard deviation obtained from three independent experiments are depicted.
truly important for the binding to the OSMR and consequently for efficient OSMR surface expression. Truncated constructs lacking the full SH2 domain or lacking the major part of the SH2 domain show a clear reduction in their ability to promote receptor surface expression. This finding is in accordance with data published for the EpoR and the IFNα receptor. In both cases, only constructs (Jak2 in case of the EpoR and Tyk2 in case of the IFNAR1 chain) comprising an intact SH2 domain are also able to support efficient surface expression of the bound receptors (5, 7). In these cases, however, deletion of the SH2 domain did not affect binding to the receptors. This is clearly different in case of the OSMR: partial or full deletion of the SH2 domain leads to constructs that bind to a much lesser extent to the chimeric OSMR than the construct containing the intact SH2 domain (Fig. 5A). This implies that in the case of the OSMR/Jak1 interaction, the FERM domain by itself is not sufficient for high affinity receptor binding. In case of gp130, it was shown by the use of chimeric constructs of Jak1 and Jak3 that the FERM domain is sufficient for binding (12). We also observed that the FERM construct of Jak1 binds to gp130 (Ref. 11 and data not shown). Thus, the structural requirements for receptor interaction might vary between different receptor systems. Interestingly, we found that in the case of the binding of Jak1 to the OSMR, the SH2 domain of Jak2, but not the SH2 domain of Jak3, is able to compensate for a missing Jak1 SH2 domain.

The role of the SH2 domain may be to anchor the FERM domain in a binding-competent state, which would imply that it is very unlikely that the FERM and SH2 domains exist as independent entities. We rather hypothesize that there are some structural interactions between these domains which could explain our finding that the SH2 domain of Jak2 but not Jak3 can substitute for the SH2 domain of Jak1 in OSMR up-regulation.

Further support for the structural role of the Jak SH2 domain can be deduced from the alignment shown in Fig. 1. Interestingly, the extremely well conserved tryptophan and tyrosine residues (conserved to greater than 90% in SH2 domains) in the β-strand cannot be found in any of the Jak sequences. The tryptophan residue normally anchors the N-terminal tail at the back of the SH2 domain and directs it away from the phosphotyrosine recognition site. As the C-terminus of SH2 domains is also directed toward the back of the domain, interference of N- and C-terminal domains with SH2-binding partners is prevented. The absence of the well conserved tryptophan indicates that, in case of the Jak3, the domain preceding the SH2 domain, namely the FERM domain, could be positioned not behind, but aside, the SH2 domain. Thus, the SH2 domain could more function as a spacer between its neighboring domains and as such be important for the conformation of the molecule.

Nevertheless, it cannot be excluded that the SH2 domain in Jaks still may be an interaction domain. In theory, it could bind non-classical motifs, like non-phosphorylated peptides or bind proteins by a totally different mechanism. Our attempts to pull down interaction partners with isolated SH2 domains of Jaks have so far yielded no results (data not shown). Unfortunately, the structure of Janus kinases has not been successfully explored so far as it may give valuable information about the function of the different domains.

Taken together, the present in depth study on the SH2 domain of Jaks, indicating that the SH2 domain does not fulfill a classical SH2 function, adds to the mystery of these kinases that already harbor a kinase domain with a non-classical function.

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The Jak1 SH2 Domain Does Not Fulfill a Classical SH2 Function in Jak/STAT Signaling but Plays a Structural Role for Receptor Interaction and Up-regulation of Receptor Surface Expression

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