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# The RET finger protein interacts with the hinge region of SMC3 ☆

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**Abstract**

The structural maintenance of chromosome 3 protein (SMC3) is a component of the multimeric cohesin complex that holds sister chromatids together and prevents their premature separation during mitosis. By screening a human cDNA library for interacting proteins we have established that the proto-oncogene RET finger protein (RFP) interacts with SMC3. The sites of interaction map to part of the central coiled coil region of RFP and to the C-terminal region of the SMC3 globular hinge domain. SMC3/RFP interaction was confirmed in vivo by co-immunoprecipitation studies and by performing mammalian two-hybrid interaction assays. Cytoimmunolocalization experiments showed that SMC3 and RFP co-localize in the same cell substructures. Overexpression of RFP in NIH3T3 cells significantly increased the fraction of SMC3 recovered in the nucleus supporting the idea that RFP regulates the intracellular distribution of SMC3. These studies identify a novel SMC3-interacting protein that may affect SMC3 availability to complex with its cohesin partners.


*Keywords:* SMC3; SMC1; Cohesin complex; RFP; Protein–protein interaction; Mammalian two-hybrid system, ND-10 nuclear bodies

The structural maintenance of chromosome 3 protein (SMC3, formerly called bamacan, cspg6, HCAP, SmcD, or Mmip1) [1–4] is a component of the soluble cohesin complex that is responsible for the ordered segregation of sister chromatids during cell division. In *Saccharomyces cerevisiae* the complex also includes SMC1, Scc1, and Scc3 [5–7]. Whereas native SMC3 does not form homodimers, the substitution of the SMC3 hinge with that of SMC1 leads to protein dimerization supporting the conclusion that the central globular region of the protein is necessary and sufficient for heterodimerization [8]. It has been proposed that the SMC1/SMC3 bipartite complex encircles the sister chromatids forming a ring that is sealed by Scc1 through a process mediated by the SMC proteins' ATPase head [8–11].

High transcript level of SMC3 has been detected in colon carcinomas [12], in liver metastatic cancer cells [13], in vascular endothelial cells following angiogenic stimulus [14], in marrow stem cells exiting quiescence [15], and in human T-cells infected with Varicella-Zoster [16]. On the other hand, SMC3 expression is down regulated in rat kidney epithelial cells transfected with Ha-ras [17], in lung epithelial cell infected with WSN virus [18], and in damaged axons [19]. In these contexts changes in *SMC3* transcriptional activity are not accompanied by a corresponding change in the expression of the other components of the cohesin complex. In addition, SMC3 and SMC1 are differentially expressed in several organs [3,20]. Because the different proteins of the cohesin complex combine in stoichiometric ratio, the excess SMC3 is either engaged in other protein–protein interactions or is free. In order to identify proteins that interact with SMC3 and may be involved in regulating its cellular localization and multimeric complex formation, a yeast two-hybrid system was utilized to screen

☆ *Abbreviations:* SMC3, structural maintenance of chromosome 3; RFP, RET finger protein.

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a human fetal brain cDNA library. SMC3 cDNA coding for the central domain of the protein harboring the hinge and part of the flanking coiled coil domains was used as bait. Here we report that the proto-oncogene RET finger protein (RFP) physically interacts with a restricted region of the SMC3 hinge domain and that the two proteins partly co-localize in the same nuclear subcompartment. We furthermore demonstrate that RFP is a determinant of SMC3 nuclear level.

## Materials and methods

*Plasmid constructs.* The procedures for the generation of SMC3 and SMC1 constructs in pGBKT7 or pGADT7 vectors have been described in a recent publication [21]. Full-length human RFP cDNA was generated by RT-PCR from RNA isolated from 293 cells using the primers 5′-CCGGCGCCATGGCCTCCGGGA-3′ and 5′-AGGGGAGGTCTC CATGGAATGACCATGATTC-3′ and cloned in pcDNA3.1/V5-His. RFP deletion constructs were generated by PCR from the full-length cDNA template using primers terminated with restriction sites useful for cloning into pGADT7. For RFP-1/246 the primers used were: 5′-CCGGCGCCATGGCCTCCGGGA-3′ and 5′-CTAGAATT CGCCATCTACAATAGCATCATGGT-3′. RFP-1/131 was obtained by digesting pGADT7-RFP-1/246 with *Xho*I and ligation of the internally deleted vector. RFP-210/366 and RFP-366/514 were obtained by priming with 5′-CTAGAATTCGCCATCTACAATAGCATCATGG T-3′ and 5′-TGGGGATCCCAAGACACAGGGAAAGAGAATTG-3′ or 5′-TGTGAATTCGGCTCTCCATGCTTCATCGCC-3′ and 5′-A GGGGATCCCTCCATGGAATGACCATGATTCCC-3′, respectively. RFP-210/514 in pGBKT7 was generated by retrieving the insert from the pACT2 plasmid identified by two-hybrid library screening of the human cDNA library.

*Yeast two-hybrid cDNA library screening.* AH109 yeast transformed with SMC3-465/807 acting as bait was mated with Y187 yeast pre-transformed with a human fetal brain cDNA library in pACT2 vector according to the manufacturer's protocol (Clontech). After selection on SD-Ade/-His/-Leu/-Trp plates, cDNA encoding the interacting polypeptides was isolated from the positive colonies and sequenced. To identify the SMC3 and RFP interacting regions, AH109 was co-transformed with truncation mutants of SMC3 in pGBKT7 and of RFP in pGADT7. Positive interactions were assessed by growth on SD-Ade/-His/-Leu/-Trp/X-α-Gal plates. To evaluate the strength of interaction, three colonies were randomly selected and grown overnight in selection media. Cells were lysed and β-galactosidase activity was assessed using ONPG (1 mM) as substrate in the presence of 50 mM β-mercaptoethanol. After 2 h incubation at 37 °C, the color developed was read at $OD_{420}$.

*Mammalian two-hybrid interaction assay.* Linear cDNA constructs expressing the bait and prey fusion proteins were generated using the Verve mammalian two-hybrid kit (Invitrogen). The cDNA inserts were generated by PCR using adapter primers according to the manufacturer's instruction. The bait and prey DNA constructs together with a reporter plasmid (pGAL/lacZ) were co-transfected into 293 cells using Lipofectamine. Cells were harvested after 48 h and the strength of the protein–protein interaction was assayed with a β-galactosidase luminescence kit (Invitrogen).

*Protein complex immunoprecipitation.* SMC3/RFP complexes were immunoprecipitated from lysates for 293 cells. Cultures grown at ~80% confluence in 10 cm plates were rapidly washed in ice-cold phosphate-buffered saline and lysed in 2 ml of 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40/0.5% Na-deoxycholate buffer containing 100 mM NaF, 2 mM $Na_3VO_4$, 10 mM phenylmethylsulfonyl fluoride, 500 μM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 150 nM aprotinin, and 1 μM leupeptin. The lysate was centrifuged at 12,000*g* and the supernatant was preabsorbed on protein G–agarose beads. One milliter aliquots of the cell lysate were then incubated overnight at 4 °C with 50 μg SMC3 antibody (Santa Cruz Biotech) and the immunocomplexes were captured on protein G–agarose bead. After recovery by centrifugation, the agarose beads were washed twice with 1 ml of 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40/0.5% sodium deoxycholate buffer, and once with 10 mM Tris–HCl, pH 7.5, 0.1% Nonidet P40/0.05% sodium deoxycholate buffer. After solubilization in SDS-containing sample buffer, the proteins were analyzed by 8% SDS–PAGE and transferred to nitrocellulose membranes by electroblotting. The filters were then blocked in 5% BSA for 18 h at 4 °C and probed with rabbit anti-human RFP antibody (IBL America) (200 ng/ml) for 1 h at room temperature. After incubation with anti-rabbit IgG HRP-conjugated secondary antibodies (1:10,000), the immunocomplexes were visualized by ECL. in vitro SMC3/RFP interaction was investigated using [$^{35}$S]methionine-labeled full-length SMC3 and RFP. For this purpose the corresponding expression plasmids were transcribed and translated in vitro using a TnT coupled reticulocyte lysate systems (Promega) in the presence 10 μCi of [$^{35}$S]methionine. The reactions were performed at 30 °C and stopped after 120 min. In order to confirm the product identity and to assess the labeling efficiency, the translated proteins were analyzed by 10% SDS–PAGE. To detect the formation of SMC3/RFP complexes, 25 μl aliquots of the SMC3 and RFP TnT cocktails were combined and incubated at room temperature for 1 h to allow protein heterodimerization. The complexes formed were immunoprecipitated with anti-SMC3 (5 μg) or alternatively anti-RFP antibody (2 μg), captured on protein G–agarose by incubation at 4 °C overnight, and analyzed by SDS–PAGE followed by autoradiography.

*Tissue immunohistochemistry of SMC3 and RFP.* Archival paraffin blocks of tumoral tissue of five patients with breast invasive carcinoma were obtained from the surgical pathology service of the Jefferson Hospital. For immunohistochemistry, serial sections of 5 μm were subjected to antigen retrieval by microwaving in 0.1 M citrate buffer, pH 6.0, for 10 min. Sections were then incubated for 1 h with either goat anti-SMC3 (1:200) or rabbit anti-RFP (1:200). The immunocomplexes were visualized using Vecstatin ABC kit and a multilink horseradish peroxidase-conjugated secondary antibody (Vectors Labs). All the operations were carried out in a Biogenex Optimax Autostainer. Mounted slides were examined and photographed on a Zeiss Axioskop 2 light microscope.

*Cytoimmunolocalization of SMC3 and RFP.* HeLa and NIH/3T3 cells grown on poly-lysine-coated coverslips were fixed in 4% paraformaldehyde for 5 min at room temperature and permeabilized for 5 min in PBS/0.1% Triton X-100. After washing the cells were blocked with 1% BSA in PBS at room temperature for 1 h. For indirect immunofluorescence of RFP and SMC3, the cells were incubated 1 h with the primary antibodies (1:200), washed three times for 5 min with PBS, and incubated with chicken Alexa488-conjugated anti-goat IgG (1:100) and chicken Alexa546-conjugated anti-rabbit IgG (1:100) (Molecular Probes). After 1 h incubation, the cells were washed and treated with Antifade reagent. Slides were mounted in Gel/Mount and observed under a BioRad MCR 600 confocal microscope.

*Cell subfractionation.* Stepwise separation of cell cytoplasmic and nuclear protein extracts was carried out using a NE-PER extraction reagents kit (Pierce) in the presence of protease and phosphatase inhibitors (see above). An aliquot (50 μg as protein) of the cell fractions was analyzed on 10% SDS–PAGE, and RFP, SMC3, and Lamin (as nuclear protein marker) were identified by Western immunoblotting as described. 293 cells stably overexpressing RFP were generated by transfection with 3 μg pcDNA3.1-RFP vector. The control cell line was generated by transfection with pcDNA3.1 vector alone. After selection in medium containing 500 μg/ml G418, the surviving cell clones were expanded individually and RFP expression was examined by semiquantitative RT-PCR or by Western immunoblotting.

## Results

### *Yeast two-hybrid screening identifies RFP as an SMC3 interacting-protein*

In order to identify proteins interacting with the hinge globular domain of SMC3, a bait construct was generated by cloning the central region of SMC3 coding sequence in pGBKT7 for expression as a GAL4 DNA binding fusion protein. The bait encompasses the full hinge domain of the protein (161 amino acids), and is flanked at the N- and C-termini by 41 amino acids and a 140 amino acid coiled coil segment, respectively. The bait named SMC3-465/807 (see Fig. 1 for a diagram

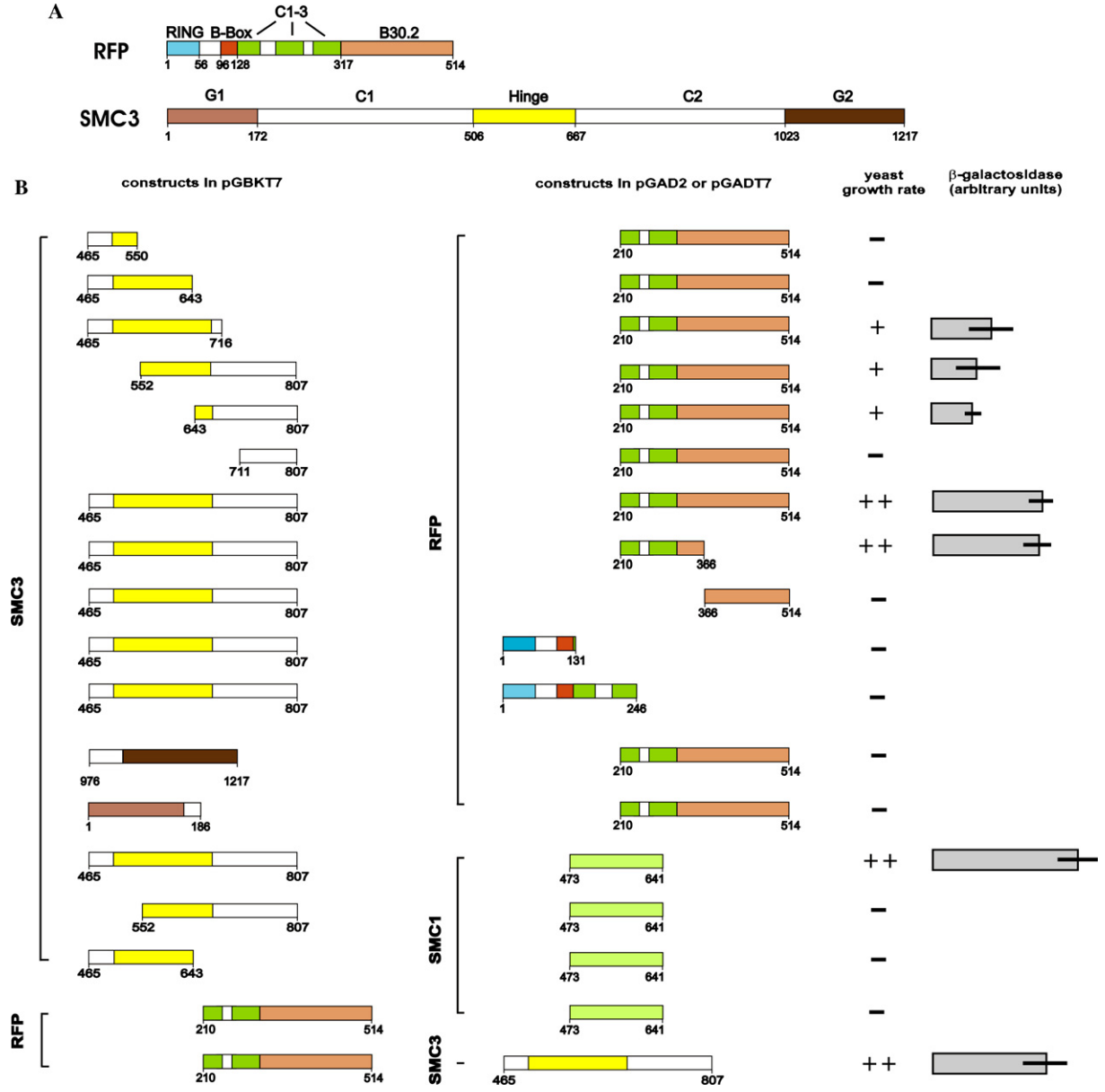


Fig. 1. (A) RFP and SMC3 structural domains. RFP: the RING, B-box, the coiled coil (C1 to C3), and the B30.2 (also known as rfp) domains are illustrated. SMC3: the three globular domains are identified as G1, G2, and Hinge, respectively, whereas the coiled coil domains are named C1 and C2. (B) Constructs utilized in the two-hybrid assay. All numerals refer to the amino acid sequence. RFP-210/514 represents the protein coding sequence contained in the pACT2 plasmid retrieved from the screening of the Matchmaker cDNA library. SMC3-465/807 harbors the protein hinge domain and after cloning in pGBKT7 was utilized as bait for the screening of the Matchmaker cDNA library. The same insert was also cloned in pGADT7. SMC1-473/641 spans the hinge domain of the protein. In order to assess the strength of protein–protein interaction, yeast cells were seeded on SD-Ade/-His/-Leu/-Trp/X-α-Gal selection media plates and colony growth was scored. The highest score (++) was assigned to colonies with growth rate similar to those observed for pGAD-SV40 large-T antigen and pGBKT7-p53 co-transformants used in the assay as a positive control. The null score (−) was assigned when no colonies were visible after 10 days. The co-transformation efficiency was assessed by plating on SD-Leu/-Trp media. β-Galactosidase activity expressed as a result of the protein–protein interaction was assessed using a colorimetric assay. The data shown were normalized for the time of incubation with the substrate and the amount of yeast cells ($OD_{600nm}$). The bars represent the average ± SD values from three independent yeast cultures.

of the constructs used and their designation) was used for the screening of a pre-transformed human fetal brain Matchmaker two-hybrid library (Clontech) cloned in the pACT2 and expressing GAL4 transactivation domain fusion proteins. Forty positive colonies reaching 2 mm size after 1 week were collected and 21 of the isolated plasmids with inserts greater than 500 bp were sequenced. Two of the sequences matched ~1.5 kb of the same region of RFP cDNA (GenBank BC013580) including ~600 bp of the gene 3′-UTR.

*Mapping of the RFP and SMC3 interacting regions*

*RFP* encodes a 514 amino acid polypeptide including a globular RING finger, a B-box zinc finger, a coiled coil domain composed of three helices, and a C-terminal region called B30.2 or rfp domain (Fig. 1). The RFP clone retrieved from the human library (named RFP-210/514) harbors the C2–C3 coiled coil domains and the B30.2 domain of the mature protein. Yeast co-transformed with SMC3-465/807 and RFP-210/514 or RFP-210/366 grew rapidly. On the contrary RFP-366/514, RFP-1/246 or RFP-1/131 co-transformants did not grow. Switching RFP-210/366 to the pGBKT7 vector and SMC3-465/807 to pGADT7 confirmed the previous results. The rate of binding between RFP and SMC3 was measured by monitoring the β-galactosidase expressed as a result of the protein–protein interaction and found comparable to that between SMC1-473/641 (encoding the hinge region of SMC1) and the SMC3 bait. In order to confirm these results, the interaction between RFP and SMC3 was investigated using a mammalian two-hybrid in vivo interaction assay. For this purpose 293 cells were co-transfected with an SV40 promoter-driven SMC3-465/807 linear vector and various RFP constructs either encoding the entire protein, the RFP sequence identified in library screening or a non-interacting region of the protein (Fig. 2A). The results from this series of experiments indicate that the RFP region spanning the $Glu^{242}$ to $Cys^{366}$ region and comprising the entire C3 coiled coil segment and part of the B30.2 domain is required for binding to SMC3. To identify the region of SMC3 responsible for interaction with RFP, a series of truncation mutants of SMC3 were tested. SMC3-552/807, SMC3-643/807, and SMC3-465/716 co-transformants with the RFP prey vector (RFP-210/514) gave colonies growing at the same rate that as observed with the full bait. On the contrary, SMC3-465/550, SMC3-465/643, and SMC3-711/807 co-transfectants produced no colonies. Additional experiments carried out to examine the possibility that RFP interacts with the terminal globular regions of SMC3 (SMC3-1/186 and SMC3-976/1217) also gave negative results. Based on these findings we conclude that the SMC3 region spanning $Ala^{643}$ and $Asp^{716}$, and comprising the C-terminal region of the SMC3 hinge domain is mediating the interaction with RFP. No interaction, on the other hand, was identified between RFP-210/514 and the hinge region of SMC1 (SMC1-473/641).

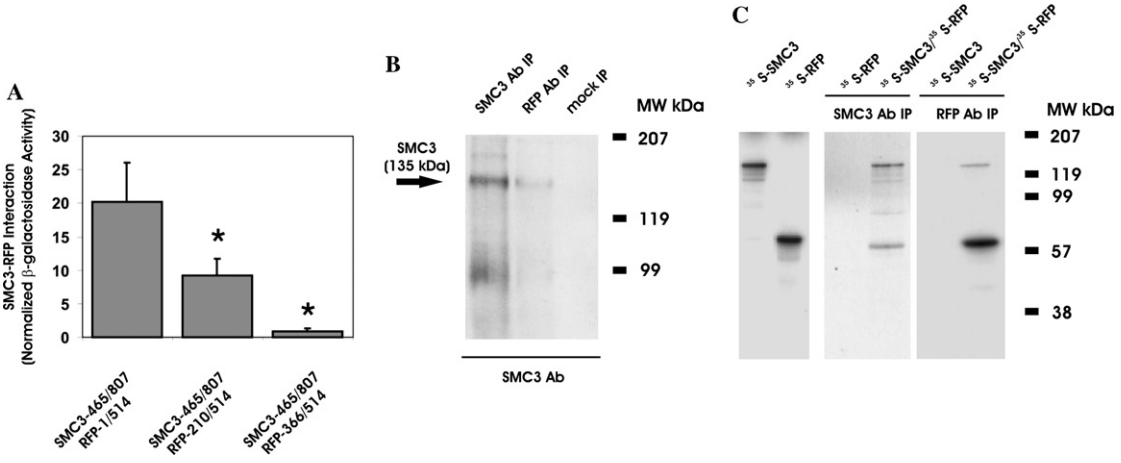


Fig. 2. SMC3 and *RFP* in vivo interaction and co-immunoprecipitation. (A) The strength of interaction between SMC3 and different regions of RFP was investigated in 293 cells using a mammalian two-hybrid system. Cells were co-transfected with a linear construct harboring the SMC3-465/807 insert and with different RPF-deletion constructs as indicated. After 48 h incubation, the cells were harvested and the expressed β-galactosidase activity measured using a luciferase-based assay. (B) Co-immunoprecipitation and Western immunoblotting of RFP and SMC3 from cell lysates. SMC3-or RFP-antibody immunoprecipitated material originated from the same cell lysate and captured on agarose–protein G were electrophoresed on 8% SDS–PAGE. Protein bands were visualized by ECL using HRP-conjugated secondary antibodies. (C) [$^{35}$S]Mthionine-labeled RFP and SMC3 were generated by in vitro coupled transcription–translation using full-length cDNA clones in pcDNA-3.1. To confirm the identity of the labeled products, samples of the reticulocyte lysates were directly analyzed on 10% SDS–PAGE. For the co-immunoprecipitation reaction, aliquots of either $^{35}$S-SMC3 or $^{35}$S-RFP either alone or together were incubated with anti-RFP or anti-SMC3 antibody at 4 °C for 18 h followed by absorption of the immunocomplexes on agarose–protein G beads. After electrophoresis bands were visualized by autoradiography.

*RFP and SMC3 form complexes in vivo*

Analysis of the RFP immunoprecipitate from the lysate revealed the presence of SMC3 (Fig. 2B). Based on the SMC3 immunoreactivity in the SMC3 and RFP immunoprecipitates from the same volume of lysate, we estimate that up to one-fifth of SMC3 present in 293 cells is available for interaction with RFP. Because of the similar size of RFP and of the precipitating immunoglobulins, no definitive conclusion could however be reached about the amount of RFP present in the SMC3 immunoprecipitate. To circumvent this problem and at the same time to examine whether direct interaction (i.e., not mediated by linking proteins) is responsible for the co-immunoprecipitation of the two proteins, the ability of RFP and SMC3 to form dimeric complexes was tested in vitro. For this purpose [$^{35}$S]methionine-labeled full-length proteins were generated using a rabbit reticulocyte lysate system. SDS–PAGE analysis demonstrated that transcripts of the expected size had been obtained (Fig. 2C). For the co-immunoprecipitation experiments, the reticulocyte lysate cocktails containing the labeled transcripts were incubated with either anti-SMC3 or anti-RFP antibody. Control experiments performed with a single labeled protein established that the antibodies did not cross-react and that aspecific binding of the proteins to the agarose-G beads was negligible. The analysis of the immunocomplexes formed in samples containing both labeled RFP and SMC3, revealed the presence of RFP in the SMC3 immunoprecipitate and of SMC3 in the RFP immunoprecipitate (Fig. 2C), supporting the conclusion that SMC3 and RFP are capable of direct interaction.

*RFP and SMC3 display similar staining pattern in breast tissue sections*

To further investigate the nature of the interaction between RFP and SMC3, immunohistochemistry analysis was performed on human tissue sections. RFP is expressed at relatively low level in most tissues with maximal expression detected in the testis and the brain. By querying the SAGE database we found that the gene is differentially expressed in several breast carcinoma cDNA libraries. Five serial specimens from patients undergoing surgery for invasive carcinoma were stained with anti-RFP or anti-SMC3 antibodies. RFP and SMC3 were mainly detected in the nuclei of the invading cells (Figs. 3A and B). In all the samples examined, RFP immunoreactivity had an uneven and speckled appearance as previously described for this antigen in tissue sections and cultured cells [22]. SMC3 displayed a similar staining pattern.

*SMC3 and RFP co-localize within the cell*

To further examine RFP and SMC3 association in vivo, immunofluorescence studies were performed in

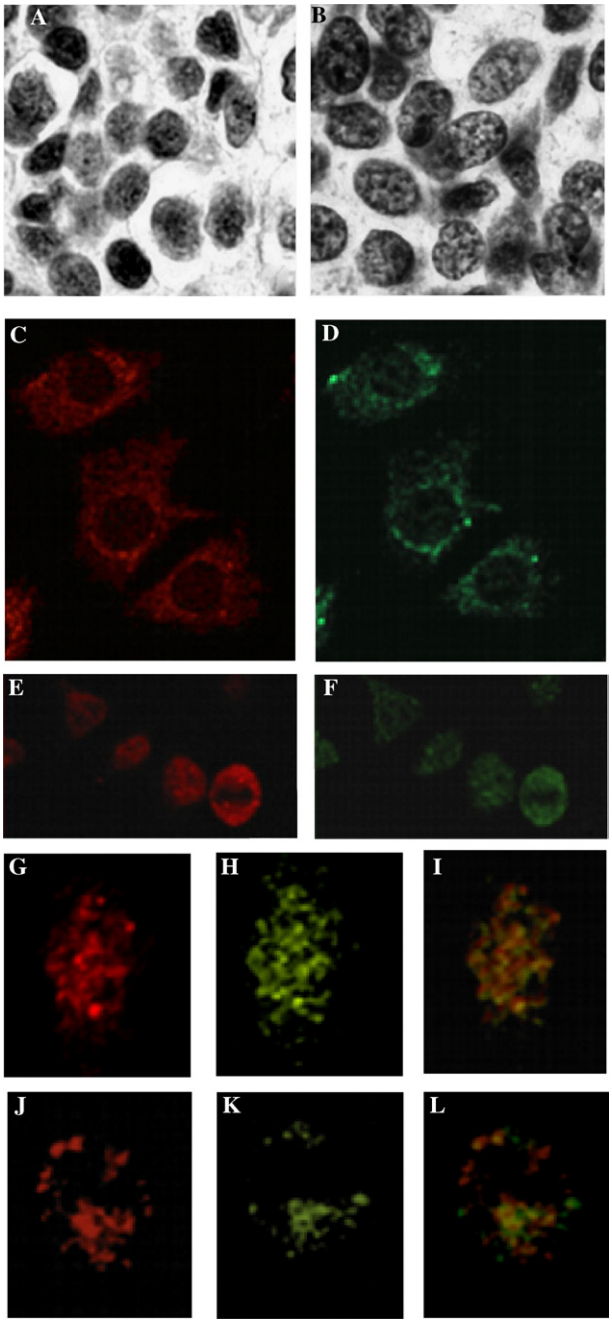


Fig. 3. Cytolocalization of SMC3 and RFP. (A,B) Immunohistochemical analysis of SMC3 and RPF in invasive breast carcinomas. Serial sections of tissue were incubated for 1 h with either rabbit anti-RFP (A) or goat anti-SMC3 (B) antibodies and the immunocomplexes were visualized using HRP-conjugated secondary antibodies. Note the speckled staining pattern of the RFP and SMC3 antigens in the nucleus of the cells. (C–L) Cytolocalization by immunofluorescent analysis of SMC3 and RFP in HeLa and NIH-3T3 cells. RFP was detected with a rabbit primary antibody and Alexa546-conjugated anti-rabbit IgG (red) (C,E,G,J). SMC3 was detected by direct immunofluorescence staining using a goat primary antibody indirectly labeled with Alexa488-conjugated anti-goat IgG (green) (D,F,H,K). Both SMC3 and RFP are mainly detected in the cytoplasm of NIH-3T3 cells (C,D). In HeLa cells (E,L) the two antigens are predominantly found in the nucleus where they co-localize in nuclear bodies. Areas of co-localization appear in yellow in (I) and (L) following the merging of the red and green confocal microscopy images shown in (G,H) and in (J,K), respectively.

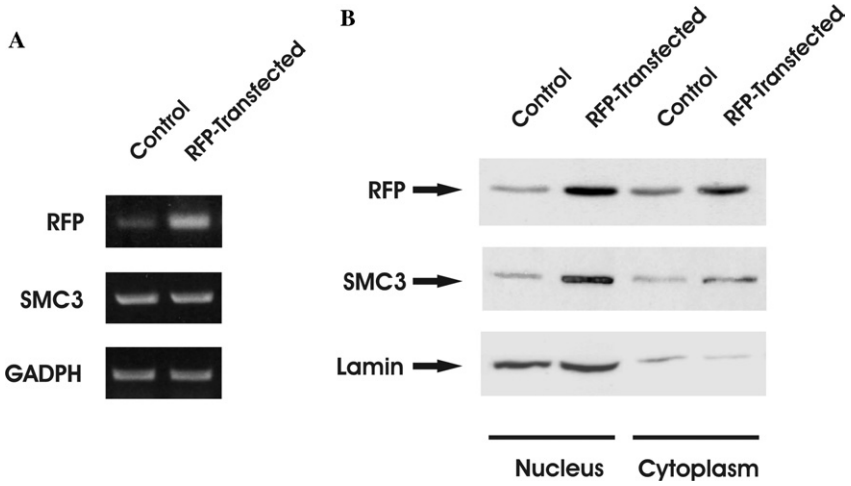


Fig. 4. Effect of RFP overexpression on the intracellular localization of RFP and SMC3. (A) Transcript level of SMC3, RFP, and GAPDH in cells stably overexpressing RFP and in control cells (expressing the drug selection gene alone). Single stranded cDNA was generated by reverse transcription and amplified by RCR using gene-specific primers. Agarose bands were visualized with ethidium bromide and their intensity analyzed by scanning densitometry. (B) Subcellular localization of SMC3 and RFP. Cell nuclear and cytoplasmic fractions were obtained by stepwise extraction with detergents, and the proteins were analyzed by Western immunoblotting after protein separation on 10% SDS–PAGE. Lamin A/C was used as nuclear marker and detected with a monoclonal antibody (Santa Cruz Biotech).

two cell lines with different RFP intracellular distribution. In NIH/3T3 cells, RFP is expressed as fusion protein with the RET tyrosine kinase domain and is largely localized in the cytoplasm [22]. In these cells we found that SMC3 is localized both in the nucleus as well as in the cytoplasm (Figs. 3C and D). In HeLa cells, on the other hand, RFP is almost exclusively detected in the nucleus where displays a characteristic speckled pattern (Fig. 3E). By and large SMC3 was also localized in the nucleus of HeLa cells and, as RFP, the antigen had a punctuate appearance (Fig. 3F). The superposition of the confocal microscopy images from HeLa cells revealed the concomitant presence of RFP and SMC3 (Figs. 3G–L) in several areas supporting the conclusion that RFP and SMC3 interactions are maximal at discrete sites within the nucleus. In order to examine whether overexpression of RFP affects the intracellular level and the distribution of SMC3, a clone of 293 cells stably overexpressing RFP were generated. Nuclear and cytoplasmic extracts were obtained from confluent cultures. Cells overexpressing RFP displayed a 5-fold increase in RPF transcript but SMC3 transcript was not significantly affected (Fig. 4A). Excess RFP protein was mainly localized in the nucleus and this increase was matched by a dramatic elevation of SMC3 at the same site (Fig. 4B).

## Discussion

In mammalian cells, the interference with the mechanism of cohesin assembly and dissolution can trigger chromosomal instability [11,23]. Furthermore, overexpression of SMC3 leads to cell transformation [12] suggesting that the maintenance of the exact dosage of the different cohesin complex components is critical for cell growth and differentiation. In addition to the mechanism affecting SMC3 expression and protein level, SMC3 function may also be altered through interaction with proteins that direct its intracellular localization or alternatively affect its availability to complex with the other cohesin components. In order to investigate this issue, in this study a region of SMC3 implicated in the cohesin complex formation was used as bait for the screening of a library of interacting proteins. This has allowed us to identify RFP as a SMC3-interacting protein and their direct interaction has been subsequently confirmed through a series of complementary experiments. SMC3 binding to RFP is mediated by a short segment of the protein hinge domain that overlaps with the binding region for SMC1. We show that RFP and SMC3 co-localize within the same nuclear bodies and that RFP overexpression enhances SMC3 presence in the cell nucleus. These findings support the idea that RFP may be an important determinant of SMC3 function and intracellular distribution.

RFP has been first identified as part of the human recombined transforming gene *ret* [24] and is a member of the tripartite motif (TRIM) protein family [25–27]. It has been postulated that multimeric complexes made of TRIM proteins define different subcellular compartments [22]. Depending on cell type and tissue, RFP is localized either to the cytoplasm or nucleus. The localization is dependent upon the presence of a nuclear export signal (NES) sequence located in the N-terminal region of the coiled coil domain [28]. In this study, we have determined that the SMC3 interaction site is har-

bored in a different region spanning 80 amino acids at the C-terminal of the RFP coiled coil domain. This same region has been previously shown to be required for the homodimerization of RFP and is the site for interaction with other proteins [22]. One of these proteins is PML (a tumor suppressor implicated in leukemia), and is a main component and the organizer of a subset of nuclear bodies known as PML nuclear bodies or nuclear domain 10 (ND10) [29]. Under light microscope, ND10 bodies appear as speckled structures that are present in almost all mammalian cells and are localized between condensed chromosomes [25]. The finding that SMC3 and RFP display the same staining pattern in tissue specimens and co-localize within the same cell substructures supports the conclusion that SMC3 is also a constituent of the ND10 bodies. These structures store several proteins relevant for tumorigenesis including p53, Mdm2, BRCA1, the retinoblastoma gene product Rb, Mi-2β, and Int-6 in addition to RFP [30–33]. Interestingly, a BRCA1/SMC1/SMC3 complex has been recently identified and plays a key role as effector of the ATM/NBS1 DNA-damage surveillance pathway [34]. RFP also physically interacts with Mi-2β [33], a protein that together with Scc1 is part of the human chromatin-remodeling complex that is responsible for the loading of cohesin onto human chromosomes [35]. Thus, RFP-containing nuclear bodies store at least two other SMC3-interacting proteins, suggesting that they may be a site of organization of SMC3-containing multimeric protein complexes.

The finding that SMC3 is present in the cytoplasm of NIH/3T3 cells while is found almost exclusively in the nucleus of HeLa cells mirroring the intracellular distribution of RFP in these cells indicate that binding to RFP may be relevant for the intracellular fate of SMC3. This idea is further corroborated by the evidence that SMC3 is increased in the nucleus of cells overexpressing RFP. This mechanism would be analogous to what was previously described for the proto-oncogene Int-6 whose translocation from the cytoplasm to the ND-10 nuclear bodies is triggered by RFP [32]. At the nuclear level RFP may influence the rate of heterodimerization of SMC3 with SMC1 by changing SMC3 availability. In addition, as recently demonstrated for the SMC3-interacting protein Hinderin [21], RFP may act as SMC1 competitor. The association of SMC3 with proteins affecting its intracellular localization and modulating its interaction with the other cohesin partners points to a novel level of regulation of the cohesin complex formation.

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