The RING Finger Protein RNF4, a Co-regulator of Transcription, Interacts with the TRPS1 Transcription Factor*

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The TRPS1 gene encodes a repressor of GATA-mediated transcription. Mutations in this gene cause the tricho-rhino-phalangeal syndromes, but the affected pathways are unknown. In a yeast two-hybrid screen with the C-terminal part of the murine Trps1 protein as bait, we obtained three yeast clones encoding two overlapping fragments of the 194 amino acids RING finger protein 4 (Rnf4). The overlap narrows down the Tnps1-binding region within Rnf4 to amino acids 6–65. This region in Rnf4 is also known to interact with several proteins including steroid receptors. By using truncated Trps1 constructs, the Rnf4-binding region in Trps1 could be assigned to amino acids 985–1184 of 1281. This 200 amino acid region of Trps1 does not contain any predicted protein-protein interacting motif. Complex formation between the human proteins TRPS1 and RNF4 was verified by co-immunoprecipitation from transfected and native mammalian cells. Confocal laser-scanning microscopy revealed that the endogenous proteins are located in distinct structures of the nucleus. Using a luciferase reporter assay, we could demonstrate that the repressional function of TRPS1 is inhibited by RNF4. This finding suggests that RNF4 is a negative regulator of TRPS1 activity.

Mutations in or complete deletions of the TRPS1 gene on human chromosome 8q24.1 cause the tricho-rhino-phalangeal syndromes, which are characterized by skeletal abnormalities, sparse scalp hair, and facial dysmorphism (1). In accordance with the clinical findings, TRPS1 is predominantly expressed in the cartilage condensations, the developing joints, the hair follicles, and in the developing snout of the mouse (2, 3). Expression was also detected in many other embryonal and adult tissues (1–4). Interestingly, Chang et al. (4, 5) found that transcription of TRPS1, which they called GC79, is down-regulated by androgen in an androgen-dependent human prostate cancer cell line. Upon castration-induced androgen withdrawal, TRPS1 is up-regulated in the rat ventral prostate and is associated with apoptotic death of these cells (4). These findings suggest a role of TRPS1 in the development of androgen-independent prostate cancer, although there is no evidence for an increased risk of prostate cancer in patients with the tricho-rhino-phalangeal syndrome.

The human and mouse TRPS1/Trps1 proteins are highly similar and have an unusual combination of different zinc-finger motifs (Fig. 1A) including a GATA-type and IKAROS-like zinc fingers (1). Malik et al. (2) have shown that TRPS1 is a repressor of GATA-factor mediated gene transcription. The IKAROS-like C2H2 zinc fingers of TRPS1 constitute a dimerization domain (6). The latter zinc fingers (as well as the zinc fingers 4–6) are possibly also involved in the binding of the dynein light chain 8 (LC8a), which we have recently identified as a TRPS1-binding partner in a yeast-two-hybrid screen (7). LC8a decreases the repressional function of TRPS1 (7). It is involved in various intracellular processes of which Bcl-2-regulated apoptosis may be relevant for the pathogenesis of the tricho-rhino-phalangeal syndrome. In addition to LC8a, other putative binding partners of TRPS1 were identified. Here we report that TRPS1 interacts with the RING finger protein RNF4, which is a co-regulator of several different gene transcription mechanisms (8–12). For instance, RNF4 interacts with the androgen receptor and functions as a co-activator of androgen-mediated transcription (8). Our findings suggest that RNF4 may modulate TRPS1 transcription and the function of the gene product.

EXPERIMENTAL PROCEDURES

β-Galactosidase Assay—DNA sequences encoding amino acids (aa)1 6–65 of Rnf4, which are responsible for the interaction with Trps1, were inserted in-frame into a galactose-inducible VP16 vector (p15GALNVP0) and co-transfected with truncated Trps1 fragments fused in-frame to the LexA DNA-binding domain. The truncated Trps1 fragments (F6, F9, and F10) were generated by PCR using the plasmid pLexA-Trps1-F5 as template and inserted into the bait vector. Transfected L40 yeast cells were grown in selective medium to an A600 of 0.4–0.5, precipitated, and resuspended in 1.2 ml of buffer Z (60 mM NaHPO4, 40 mM Na2HPO4, 10 mM KCl, 1 mM MgSO4, 50 mM 2-mercaptoethanol, pH 7.0). From this suspension, 100–150 µl (VZ) were diluted to 1 ml with buffer Z and cells were lysed by the addition of 15 µl of chloroform and 10 µl of 0.1% (w/v) SDS. The mixture was equilibrated at 30 °C for a few minutes, and the reaction was started by adding 200 µl of ONPG (4 mg/ml o-nitrophenol galactose in 0.1 M K2PO4, pH 7.0). After a defined reaction period (e.g. tR = 1–3 h), the reaction was stopped by the addition of 500 µl of 1 M Na2CO3. The reaction was cleared of insoluble material by centrifugation, and the optical density was measured at 420 and 550 nm. The β-galactosidase activity was calculated in units (U) by the equation U = 1000 × A420 / 1750 × A600 × VZ × tR (13).

Co-immunoprecipitation—For immunochemical analysis, the entire coding region of the human TRPS1 was inserted in-frame into the green fluorescent protein (GFP) expression vector pEGFP-N3 (Clontech). The
full-length human RNF4 cDNA amplified from a fetal brain Marathon-ready cDNA library (catalog number 7402-1, Clontech) (5’-CCCCAACCGTTTACATGATTCAACAAAGCC-3’, 5’-CGGAATCTATATATAATTGGGTTTGTAC-3’) was cloned in-frame into a FLAG expression plasmid (pFLAG-N3). COS-1 cells were co-transfected with both plasmids by electroporation. For immunoprecipitation analysis using the lysate of transfected COS-1 cells, a volume equivalent to 100 μg of protein was dissolved in 1 ml of Dignam D buffer (20 mM HEPEs, pH 7.9, 20% (v/v) glycerol, 0.1% (v/v) Nonidet P-40, 75 mM NaCl, 100 mM KCl, 0.2 mM EDTA, 3% (w/v) bovine serum albumin). For the studies on endogenous level, a volume equivalent to 1 mg of total protein from primary human fibroblast cell extracts was dissolved in 1 ml of incubation buffer (20 mM HEPEs, pH 7.9, 75 mM KCl, 2.5 mM MgCl2, 1 mM dithiothreitol, 0.1% (v/v) Nonidet P-40, 0.5 mM phenylmethylsulfonfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 1 mM Na3VO4). The solutions were preclarified with 30 μl of preequilibrated protein A-Sepharose slurry for 30–60 min.

The supernatant of the transfected COS-1 cell extracts was then incubated with 0.2 μg of an anti-FLAG antibody (Santa Cruz Biotechnology) for 30 min and 30 μl of preequilibrated 50% (v/v) protein A-Sepharose slurry for another 90 min. To the fibroblast cell extracts, 20 μl of a monoclonal anti-TRPS1 antibody LK2B7F6 were added and incubated for 12–16 h. Subsequently, 70 μl of the preequilibrated protein A-Sepharose slurry were added and the mixture was incubated for another 90 min.

The Sepharose beads were sedimented and washed three times with incubation buffer and resuspended in 4× SDS gel-loading buffer (62 mM Tris, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.005% (v/v) bromphenol blue).

All of the incubations mentioned above were carried out at 4 °C and with constant movement using a head-over-tail rotor. The precipitates were analyzed by SDS-PAGE and Western blotting using an anti-FLAG antibody, the rabbit anti-TRPS1 antisemur SN682 (14), and a rabbit anti-RNF4 antisemur (kindly provided by J. Palvimo) (8).

Intracellular Localization—Primary human fibroblasts were directly seeded on glass coverslips. The cells were harvested after 24–48 h in cold phosphate-buffered saline, fixed with methanol, and washed with phosphate-buffered saline and with IF buffer (10 mM Tris, pH 7.5, 300 mM NaCl, 0.05% (v/v) Tween 20). In the next step, the coverslips were blocked for 30 min in 0.5% (v/v) bovine serum albumin in IF buffer, incubated with primary antibody for 1 h, washed twice with IF buffer, and again incubated with secondary antibody, and after washing with IF buffer, they were mounted on slides with AQUAMOUNT (BDH Chemicals, Product number 36086). Cell nuclei were stained with 4’,6-diamidine-2-phenylindole dihydrochloride Blue. Endogenous TRPS1 and RNF4 were detected by using rabbit antisera and stained with a purified Alexa-labeled anti-rabbit antibody. Cells were analyzed by confocal laser-scanning microscopy.

Reporter Gene Assays—Transient transfection assays including a constitutively lacZ expressing plasmid were performed in COS-7 cells essentially as described previously (2, 15). The αD3 promoter construct (provided by R. Shividassani, Boston, MA) contains multiple repeats of the sequence AGATAA upstream of the firefly luciferase cDNA. Transfections were performed using FuGENE 6 (Roche Applied Science), 0.1–0.5 μg of the αD3 reporter plasmid, 0.25 μg of the TRPS1 expression plasmid, 0.5 μg of the AGATAA expression plasmid, and 0.25–0.5 μg of the RNF4 expression plasmid. 2 μg of total DNA was used for each transfection. The individual DNA mixtures were adjusted with respective amounts of empty plasmid. After 48 h, cells were lysed at 4 °C in 25 mM Tris, pH 7.8, 2 mM 1,2-diaminocyclohexane-N,N,N’,N”-tetraacetic acid, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 2 mM dithiothreitol, 0.3 mM phenylmethylsulfonfonyl fluoride, and 2 μg/ml aprotinin. The luciferase activity was measured by luminometry (LUMAT LB9507) after dilution of the lysate 1:3 or 1:5 in 25 mM glycylglycine, 15 mM MgSO4, 15 mM K2HPO4, 4 mM EGTA, 40 μM ATP, 40 μM dithiothreitol, and 0.3 μg/ml luciferin. Relative luciferase activity was determined by comparing averaged luciferase-β-galactosidase ratios and was expressed relative to results from control transfections with the empty vector.

RESULTS

Trps1 and Rnf4 Interact in Yeast—Using a lexA yeast two-hybrid system, we have isolated 23 yeast clones expressing putative binding partners of the C-terminal fragment (aa 635–1281, Fig. 1C) of the murine Trps1 protein (7). 12 of these clones contained plasmids encoding Leu8a, which we have reported previously (7). Three other clones carried plasmids encoding two overlapping parts of the 194-aa RING finger protein Rnf4. One clone codes for an 1–130 of Rnf4, whereas the two other clones encode aa 6–65. Thus, the Trps1-binding region in the RING finger protein could be narrowed down to 60 aa of the N-terminal part (Fig. 2).

To confirm our yeast two-hybrid data and to narrow down the Rnf4 interaction region in the Trps1 protein, we performed another yeast in vivo assay. In addition to the original Trps1 bait construct (lexA-Trps1-F6), we used three other truncated fragments of Trps1 (Fig. 1C) as baits. Fragment F6 (Trps1 aa 635–1184) lacks the C-terminal IKAROS-like double zinc finger. Fragment F9 (aa 635–984) bears a larger C-terminal truncation but still includes the GATA DNA-binding zinc finger. The smallest fragment, F10 (aa 635–819), contains only the predicted zinc fingers 4–6 (Fig. 1C).

Yeast L40 cells, which carry a lacZ gene under the control of a stretch of eight lexA-operators, were co-transfected with different lexA-Trps1 fusion constructs and a galactose-inducible reporter plasmid encoding an 6–65 of the Rnf4 fused in-frame
defines the Trps1-binding region of Rnf4. It includes the NLS and the N-terminal part of the steroid receptor-interaction region.

The human RNF4 is only 190 aa in length and 90% identical to Rnf4. For all of the further investigations, we used the human orthologues TRPS1 and RNF4. The human RNF4 is only 190 aa in length and 90% identical to the mouse Rnf4 (12). The entire coding regions of TRPS1 and RNF4 were inserted in-frame into a GFP or a FLAG expression vector, respectively.

Cells co-transfected with empty GFP and RNF4-FLAG (Fig. 4B, lane 1) but not in extracts from cells where either RNF4-FLAG or TRPS1-GFP was absent (Fig. 4C, lanes 2 and 3). These findings indicate that TRPS1 and RNF4 do not only interact in yeast but are also able to form complexes in mammalian cells.

We next used primary human fibroblasts to test whether an interaction between the endogenous TRPS1 and RNF4 proteins can take place. Expression of TRPS1 and RNF4 could be readily detected in cell extracts using the anti-TRPS1 SN652 antiserum (Fig. 4D) and an anti-RNF4 antiserum (kindly provided by J. Palvimo) (Fig. 4E, lane 1) (8). To analyze the TRPS1-RNF4 interaction, a monoclonal anti-TRPS1 antibody (LK2B7F6) was successfully used to prepare immunoprecipitates from cell extracts. We were able to detect RNF4 in these precipitates with the anti-RNF4 antiserum (Fig. 4E, lane 2), demonstrating that the endogenous TRPS1 and RNF4 proteins physically interact and can be co-precipitated from human fibroblasts.

TRPS1 and RNF4 Are Located in Distinct Structures of the Nucleus—As shown previously, TRPS1 is a nuclear protein, which is located in large nuclear bodies (Fig. 5A) (see Ref. 7). The endogenous RNF4 protein has been found in the nucleus and the cytoplasm (8, 12). Because we were able to co-immunoprecipitate TRPS1 and RNF4 from human fibroblast cell extracts, we decided to evaluate the intracellular localization of both proteins within these cells. We used the rabbit anti-TRPS1 antiserum and an anti-rabbit Alexa-labeled secondary antibody to show the intracellular localization of TRPS1 (Fig. 5A). The number of TRPS1 containing nuclear bodies varied between 1 and 4 per cell. The intracellular distribution of RNF4 was investigated by using the rabbit anti-RNF4 antiserum and the Alexa-labeled anti-rabbit antibody. As shown in Fig. 5B, RNF4 is homogeneously distributed in the cytoplasm but also located in numerous small bodies in the nucleus. The RNF4-containing nuclear bodies are clearly distinct from those in which TRPS1 is found. Co-localization experiments could not be performed because both available antisera were generated in rabbit, which does not allow a specific staining of both proteins with a secondary anti-rabbit antibody.

RNF4 Inhibits the Repressional Function of TRPS1 on GATA-mediated Transcription—Using a luciferase reporter assay, Malik et al. (2) have demonstrated that TRPS1 represses GATA-dependent gene activation (2). We used this reporter assay to analyze whether RNF4 has any influence on the function of TRPS1. For this purpose, we transfected COS-7 cells with the reporter plasmid oD3, which contains the luciferase cDNA under the control of multiple AGATAA elements (15). The luciferase activity of oD3-transfected cells was arbitrarily set as 1.0 (Fig. 6, column 1). Co-transfection with the transcriptional activator XGATA4 resulted in a 5.7-fold increased activity of the reporter (Fig. 6, column 2). This activation was reduced by adding a TRPS1 expression plasmid, demonstrating the function of TRPS1 as a transcriptional repressor (Fig. 6, column 3). The TRPS1-mediated repression could be completely relieved by adding an equal amount of the RNF4 expression plasmid (Fig. 6, column 5). The addition of a higher amount of RNF4 expression plasmid resulted in an even higher
Figure 3. The Rnf4-binding region of Trps1 could be narrowed down by a β-galactosidase assay. Yeast L40 cells were co-transfected with different Trps1 constructs fused to the lexA DNA-binding domain and the galactose inducible VP16-Rnf4 prey plasmid. This yeast strain carries a lacZ gene, which is driven by a stretch of eight lexA operators. In case of an interaction of both fusion proteins, the expression of the lacZ gene is activated. A, the Trps1 fragments F5 and F6 show a strong interaction, whereas F9 and F10 do not. No signal could be detected if the constructs lexA-F5 and VP16-Rnf4 were co-transfected with the respective empty plasmids. This also excludes unspecific interactions of the lexA or the VP16 fusion tags to any of the used components. B, the Rnf4-binding-region within the Trps1 protein was narrowed down to a stretch of 200 amino acids (aa 985–1184).

DISCUSSION

In a yeast-two-hybrid screen, we have identified the RING finger protein Rnf4 as a binding partner of Trps1. Rnf4 is highly conserved in human, mouse, and rat. The rat protein was originally termed SNURF (small nuclear RING finger protein) (8). The human protein is 91% identical and is four amino acids shorter than its rodent orthologues. Mouse and rat Rnf4/SNURF share a 96% identity (8, 16). The interaction of the human TRPS1 and RNF4 was confirmed by co-immunoprecipitation of these proteins in cell extracts from transfected COS-1 cells and from primary human fibroblasts.

Transiently expressed TRPS1 and RNF4 are found exclusively in the nuclei of NIH-3T3 and COS-1 cells where they are homogeneously distributed (2, 8, 9, 11, and our data not shown). In contrast, endogenous RNF4 occurs in the cytoplasm as well as in numerous small structures within the nuclei of human fibroblasts (Fig. 5B), which was also observed by Moilanen et al. (8) in CV-1 cells. The endogenous TRPS1 protein also shows a non-homogeneous distribution within the nuclei of human fibroblasts, but the TRPS1-containing structures are less numerous and much bigger than the RNF4-containing structures (Fig. 5, A and B). This finding makes it probable that the bulk of the two proteins are involved in separate intracellular processes. Because of the lack of appropriate antibodies, we could not investigate whether the different structures overlap partially. However, our co-immunoprecipitation studies demonstrate that the two proteins do interact in human fibroblasts.

RNF4 is the second protein known to interact with TRPS1 and binds within a 200 amino acid region (aa 985–1184) of the transcription factor. It does overlap with only two base pairs with the recently identified region B of TRPS1-LC8a interaction (region A: aa 635–723; region B: aa 1182–1281) (7) and has no predicted protein-protein interaction motif.

During the last years a variety of proteins, which associate with RNF4 have been identified. RNF4 contains two distinct regions, which are known to be protein-protein-interacting domains (Fig. 2). The RING finger at the C terminus is necessary for the interaction to the TATA-binding protein (8), SPBP (11), and Sp1 (9). The second protein-interacting domain to which TRPS1 (Fig. 2) binds lies in the N-terminal part of RNF4. This region binds transcriptional repressors such as Gsc1 (12) and POZ-AT hook-zinc finger protein (PATZ) (10) as well as steroid receptors (8). In the above mentioned interactions, RNF4 undertakes several diverse functions. It can either act as a transcriptional co-repressor (10) or as a co-activator (8, 9, 11).

In the presence of androgen, for example, RNF4 strongly activates androgen receptor (AR)-dependent transcription (8). Interestingly, TRPS1 expression is repressed in androgen-dependent human prostate cancer cells (LNCaP-FGC) by physiological androgen concentrations and repression is mediated by the AR (4, 5). Furthermore, after androgen withdrawal, the Trps1 expression in the rat ventral prostate increases significantly and is associated with the regression of the prostate and the occurrence of apoptosis. RNF4 is expressed in adult mouse, rat, and human prostate (8, 12), but it is not known whether it is expressed in the LNCaP-FGC cells. If RNF4 is involved in TRPS1 repression, we assume that this repression does not affect the TRPS1 gene directly but is mediated via other AR-RNF4-regulated proteins because RNF4 normally activates AR-dependent transcription.

Analyses by Fedele et al. (10) revealed two novel functions of RNF4. They found that RNF4 enhances basal transcription from a minimal c-myc promoter in a concentration-dependent manner. The activation was reversed to a strong co-repression when the transcriptional repressor PATZ was added (10). Because TRPS1 is a repressor of transcription, we had expected a co-repressional effect of the RNF4 binding. However, in the reporter gene assay we found a complete suppression of the repressional activity of TRPS1 upon the addition of RNF4 (Fig. 6, columns 5 and 7). Our studies revealed a so far unknown function for RNF4. Obviously, it acts as a co-activator on GATA factor-mediated gene transcription. RNF4 enhanced transcrip-
RNF4 Interacts with the TRPS1 Transcription Factor

In mammalian cells, RNF4 interacts with the TRPS1 transcription factor. This interaction was monitored by using a TRPS1-GFP fusion protein and RNF4-FLAG antibody. The interaction was shown in extracts of co-transfected COS-7 cells (lane 1). If the cells were co-transfected with TRPS1-GFP/FLAG (lane 2) or pGFP/FLAG (lane 3), no TRPS1-GFP signal was detectable. D and E, the TRPS1-RNF4 interaction could also be verified on the endogenous protein level. D, the expression of TRPS1 was shown in extracts of human fibroblasts by using the αTRPS1 antiserum. E, expression of RNF4 in human fibroblasts was detected by a RNF4 antiserum (lane 1) (8). Protein extracts of human fibroblasts were incubated with monoclonal αTRPS1 antibody (lane 2), IP-TRPS1 or an irrelevant mouse antibody (lane 3, IP-X). RNF4 could only be detected in the αTRPS1 precipitates (lane 2).

Fig. 4. Expression co-precipitation of TRPS1 and RNF4 in mammalian cells. A–C, COS-1 cells were co-transfected with the TRPS1-GFP and RNF4-FLAG or each plasmid alone with the appropriate empty plasmid. A, the TRPS1-GFP fusion protein could only be identified in the respectively transfected cells (lane 2) but not in other cells (lane 1). B, the proper expression of the RNF4-FLAG construct was monitored by using a αFLAG antibody. The RNF4-FLAG fusion protein was shown in TRPS1-GFP/RNF4-FLAG (lane 1) but not in TRPS1-GFP/pFLAG-co-transfected cells (lane 2). C, extracts of co-transfected COS-1 cells were incubated with a αFLAG antibody, and the precipitates were analyzed by a TRPS1 antiserum (SN652) (see Ref. 14). The interaction of both proteins could only be found in TRPS1-GFP/RNF4-FLAG co-transfected cells (lane 1). If the cells were co-transfected with TRPS1-GFP/pFLAG (lane 2) or pGFP/pFLAG (lane 3), no TRPS1-GFP signal was detectable. D and E, the TRPS1-RNF4 interaction could also be verified on the endogenous protein level. D, the expression of TRPS1 was shown in extracts of primary human fibroblasts by using the αTRPS1 antiserum. E, expression of RNF4 in human fibroblasts was detected by a RNF4 antiserum (lane 1) (8). Protein extracts of human fibroblasts were incubated with monoclonal αTRPS1 antibody (lane 2), IP-TRPS1 or an irrelevant mouse antibody (lane 3, IP-X). RNF4 could only be detected in the αTRPS1 precipitates (lane 2).

Fig. 5. Subcellular localization of TRPS1 and RNF4. The subcellular distribution of endogenous TRPS1 and RNF4 was analyzed in primary human fibroblasts. Both proteins were visualized by using the respective rabbit antiserum and an anti-rabbit Alexa-labeled antibody. A, the TRPS1 protein was almost exclusively located in the nucleus in dot-like structures. B, the endogenous RNF4 protein could be found in the nucleus as well as in the cytoplasm. Within the cytoplasm, it is distributed homogeneously, whereas in the nuclei it shows a granular distribution pattern.

Fig. 6. The transcriptional repressor function of TRPS1 is suppressed by RNF4. COS-7 cells were transiently transfected with plasmids encoding the d3 luciferase reporter, the XGATA4 transcription factor, the TRPS1 transcription factor, and different amounts of an RNF4 encoding plasmid. The luciferase activity of cells transfected with the reporter construct alone was set as reference (column 1). Co-transfections with XGATA4 result in an increasing activity (column 2), which was reduced by adding TRPS1 (column 3). This TRPS1-induced repression could be inhibited by adding increasing amounts of RNF4 (columns 4 and 5). RNF4 shows an activation of GATA-mediated transcription (columns 4 and 5) but does not activate the reporter expression in the absence of XGATA4 (column 8). The results are representative of at least six independent experiments.

Based on current knowledge, it seems clear that RNF4 functions as a more general co-regulator, possibly as a bridging factor participating in the coordination of the activities of multiple transcription signals of upstream factors. One of these factors may be TRPS1. However, it remains elusive whether RNF4 is involved in the pathogenesis of the tricho-rhino- phalangeal syndromes because it is not known whether RNF4 is expressed, for example, in chondrocytes or hair follicles. The TRPSs are caused by haploinsufficiency (TRPS I and TRPS II) or by a dominant negative effect of a mutant TRPS1 protein (TRPS III) (see Ref. 17.). RNF4 and TRPS1 are also expressed in many tissues, which are not obviously affected in the TRPSs. Possibly, these tissues are not as susceptible to concentration imbalances as those affected in the TRPSs.

RNF4 is similar to the previously identified multifunctional LC8a (7), a suppressor of TRPS1-mediated gene repression. But in contrast to the findings of Fedele et al. (10), Moilanen et al. (8) showed that full-length RNF4-SNURF fused to the Gal4-DNA-binding domain does not activate a minimal promoter controlled by Gal4-binding sites in yeast or mammalian cells. They consequently suggested that SNURF does not have an intrinsic transcription activation function (8). In agreement with this suggestion, we found that RNF4 is not capable of activating gene transcription from an artificial GATA promoter in the absence of XGATA4 (Fig. 6, column 8).
Analyses of the other potential TRPS1-interacting proteins will show whether there are even more suppressors of TRPS1, which interact in a developmental stage or tissue-specific manner with TRPS1 to modulate its function.

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