EMBO reports

scientific report

Sulphatase activities are regulated by the interaction of sulphatase-modifying factor 1 with SUMF2

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Sulphatases undergo a unique post-translational modification that converts a highly conserved cysteine located within their active site into formylglycine. This modification is necessary for the catalytic activities of the sulphatases, and it is generated by the protein product of sulphatase-modifying factor 1 (***SUMF1***), the gene mutated in multiple sulphatase deficiency (MSD). A paralogous gene, ***SUMF2***, was discovered through its sequence similarity to ***SUMF1***. We present evidence that SUMF2 co-localizes with SUMF1 within the endoplasmic reticulum and that the two proteins form heterodimers. SUMF1 and SUMF2 also form homodimers. In addition, SUMF2 is able to associate with the sulphatases with and without SUMF1. We have previously shown that co-transfection of ***SUMF1*** with the sulphatase complementary DNAs greatly enhances the activities of the overexpressed sulphatases. Here, we show that SUMF2 inhibits the enhancing effects of SUMF1 on sulphatases, suggesting that the SUMF1–SUMF2 interaction represents a further level of control of these sulphatase activities.

Keywords: protein–protein interactions; enzymatic activity; inborn errors of metabolism

EMBO reports advance online publication 17 June 2005;
doi:10.1038/sj.embor.7400454

INTRODUCTION

The sulphatases comprise a large family of prokaryotic and eukaryotic enzymes that catalyse the hydrolysis of ester sulphates (Hopwood & Ballabio, 2001). The importance of this family of enzymes is underscored by several human genetic disorders that are caused by deficiencies in sulphatase activities. Mutations in genes encoding sulphatases have been associated with five different types of mucopolysaccharidose (MPSII, MPSIIIA, MPSIIID, MPSIVA and MPSVI), X-linked ichthyosis, metachromatic leukodystrophy and chondrodysplasia punctata (Franco *et al*, 1995; Ballabio & Shapiro, 2001; Neufeld & Muenzer, 2001; Von Figura *et al*, 2001). Another severe disease is multiple sulphatase deficiency (MSD), in which the activity of all the sulphatases is impaired, with the affected individuals manifesting features of all the known sulphatase deficiencies (Hopwood & Ballabio, 2001).

To catalyse the hydrolysis of their natural substrates, the sulphatases must be post-translationally activated. A consensus sequence in their catalytic domain contains a cysteine that is modified into a formylglycine (FGly) within the endoplasmic reticulum (ER; Schmidt *et al*, 1995). We and others have discovered a gene, sulphatase-modifying factor 1 (*SUMF1*), that encodes the formylglycine-generating enzyme (FGE; Cosma *et al*, 2003; Dierks *et al*, 2003). Mutations in *SUMF1* have been found in all MSD patients analysed, and they result in a deficiency in sulphatase activity that is due to their impaired post-translational modification (Cosma *et al*, 2003, 2004; Dierks *et al*, 2003). The FGly is essential for the activity of the sulphatases as it contains an aldehyde hydrate that attacks the sulphate ester, which leads to the formation of the enzyme–substrate complex and to the cleavage of the sulphate residue (Schmidt *et al*, 1995). Co-transfection of *SUMF1* with sulphatase complementary DNAs in cultured cells results in enhancement of sulphatase activities (Cosma *et al*, 2003).

Sequence comparisons led to the discovery of a paralogue of *SUMF1* in the vertebrate genomes, named *SUMF2*. The primary protein sequences of SUMF1 and SUMF2 are highly similar, with the exception of the region between amino acids 303 and 351 of SUMF1 (Cosma *et al*, 2003; Dierks *et al*, 2003). A peculiarity of this region is the presence, only in SUMF1, of three regularly spaced cysteine residues that are reminiscent of the iron–sulphur proteins responsible for sulphatase modifications in bacteria (Schirmer & Kolter, 1998). It is therefore likely that this region is the catalytic domain of SUMF1.

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Received 3 January 2005; revised 12 May 2005; accepted 17 May 2005; published online 17 June 2005

SUMF1 is conserved from bacteria to man, whereas SUMF2 evolved later and is present only in vertebrates. Our working hypothesis for developing the present study was that SUMF2 can act as a modulator of SUMF1. Here, we show physical and functional interactions between SUMF1, SUMF2 and sulphatases (IDS and SGSH) that regulate the post-translational activation of the sulphatases.

RESULTS AND DISCUSSION

The sulphatases are expressed in various tissues and organs, and their activities are modulated at least at three different levels: (i) the level of expression of the sulphatase genes; (ii) the kinetics of the sulphatase proteins; and (iii) the post-translational modification mediated by SUMF1. We postulated that SUMF2 represents a further level of control of the sulphatase activities. SUMF2 is found only in vertebrates, and it shares a 48% amino-acid identity and a 62% similarity with SUMF1 (Cosma *et al*, 2003). We showed the ubiquitous expression of *SUMF2* in different tissues by northern blotting. Interestingly, we saw a comparable amount of *SUMF2* messenger RNA with respect to the *SUMF1* transcript in all the tissues analysed, with the kidney, liver and placenta showing the highest levels of both of these mRNAs (Fig 1A). The similar levels of *SUMF1* and *SUMF2* expression suggested that these two genes can be transcriptionally co-regulated. We therefore used the MSD cell line to determine whether the loss of function of SUMF1 was associated with a change in the expression levels of *SUMF2*. Real-time PCR was performed on the cDNA prepared from five independent MSD cell lines and three control wild-type human fibroblasts. Intriguingly, the level of *SUMF2* expression in the MSD cell lines was significantly lower with respect to the controls (*t*-test, $P = 0.012$; supplementary Fig 1A online). This suggested that the transcription of *SUMF2* is dependent on SUMF1.

We next analysed the glycosylation content of SUMF1 and SUMF2. Carboxy-terminus Flag-tagged SUMF1 and SUMF2 were transfected in Cos7 cells. The protein extracts were digested with Endo H and peptide-*N*-glycosidase F (PNGase F) and analysed by western blotting in an 8–16% gradient gel. Different forms of SUMF1 and SUMF2 of different masses were detectable, which possibly corresponded to different glycosylated forms and/or proteolytically cleaved products (supplementary Fig 1B online). Both SUMF1 and SUMF2 showed bands that contained a high-mannose-type oligosaccharide that was sensitive to both Endo H and PNGase F.

The high amino-acid similarity and the comparable expression levels between SUMF1 and SUMF2 prompted us to explore whether they have related functions. We first determined the localization of SUMF2 after transfection in Cos7 cells. We and others have shown that SUMF1 localizes in the ER (Cosma *et al*, 2003; Dierks *et al*, 2003), and here we show that

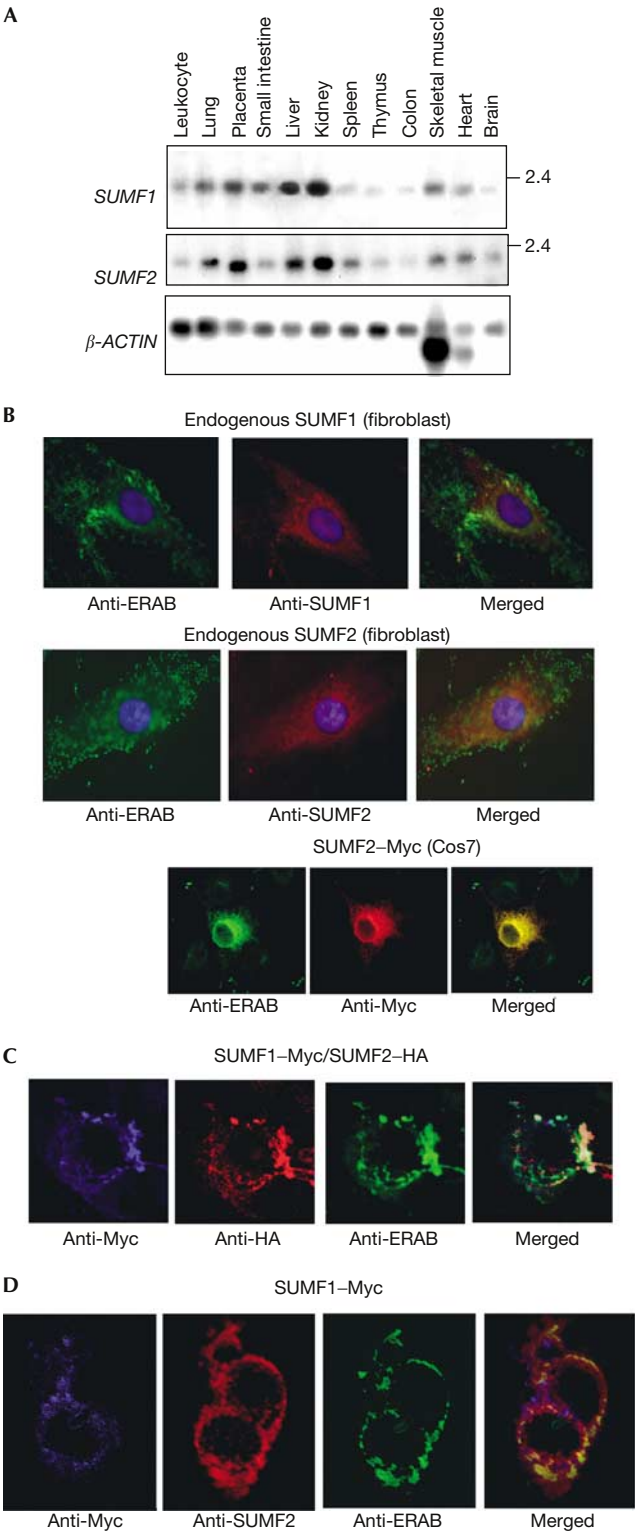

Fig 1 | Expression and cellular localization of SUMF1 and SUMF2. (**A**) Northern blotting from different tissues. The *SUMF1*, *SUMF2* and *β-ACTIN* probes were hybridized to polyA+ messenger RNA filters (Clontech, Mountain View, CA, USA). (**B–D**) Cos7 cells transfected with the indicated cDNAs were detected using the anti-Myc and anti-HA (haemagglutinin) antibodies; the endogenous proteins were detected with anti-SUMF1 and anti-SUMF2 antibodies. (**B**) Endogenous SUMF1 and SUMF2 colocalize with ERAB in the ER of fibroblasts. SUMF2–Myc colocalizes with ERAB at the confocal microscopy level. (**C**) SUMF1–Myc and SUMF2–HA colocalize with ERAB, as observed under confocal microscopy. (**D**) Colocalization of SUMF1–Myc, endogenous SUMF2 and endogenous ERAB, as observed under confocal microscopy.

endogenous SUMF1, endogenous SUMF2 and N-tagged SUMF2–Myc colocalize with ERAB, an ER marker, both in Cos7 cells and in primary fibroblasts. This was seen under both indirect and confocal microscopy (Fig 1B; supplementary Fig 2A online). Furthermore, we coexpressed the N-tagged *SUMF1–Myc* and *SUMF2–HA* (haemagglutinin) cDNAs in Cos7 cells. Again, both indirect and confocal microscopy showed that SUMF1–Myc and SUMF2–HA colocalize with ERAB in the ER (Fig 1C; supplementary Fig 2B online), and that both endogenous SUMF1 and SUMF2 colocalize with the tagged expressed proteins (supplementary Fig 2C online). Furthermore, we observed, under confocal microscopy, that SUMF1–Myc colocalizes with endogenous SUMF2 and ERAB (Fig 1D).

Following our demonstration that SUMF1 and SUMF2 co-localize in the ER, we tested whether they interact physically. For this, we co-transfected Cos7 cells with several combinations of tagged *SUMF1* and *SUMF2* cDNAs (*SUMF1–Myc* + *SUMF2–Flag*; *SUMF2–Myc* + *SUMF2–Flag*; *SUMF2–Myc* + *SUMF1–Flag*; *SUMF1–Myc* + *SUMF1–Flag*). Immunoprecipitation with the anti-Myc polyclonal antibody and immunoblotting with the anti-Flag monoclonal antibody of protein extracts from these cells showed that SUMF1 and SUMF2 interact physically, forming both homodimers and heterodimers (Fig 2A, lanes 4–7). Three bands of about 40, 39 and 33 kDa that corresponded to SUMF1 and three bands of about 33, 32 and 29 kDa that corresponded to SUMF2 were visible. No specific bands were present on immunoprecipitation of the transfected samples with an anti-HA monoclonal antibody, or with an anti-Myc antibody in nontransfected samples (Fig 2A, lanes 1–3). Western blotting of gels loaded with 10% of the total protein extracts showed the efficiency of the transfection (supplementary Fig 4A online). To confirm these interactions of SUMF1 and SUMF2, we expressed *SUMF1* and *SUMF2* cDNA hybrids for different tags in Cos7 cells. We were also able to detect the homodimer and heterodimer associations of SUMF1 and SUMF2 carrying the different epitopes (supplementary Fig 3A online). Furthermore, the overexpressed SUMF1 and SUMF2 proteins were almost entirely pulled down to form homodimers and heterodimers, as none of the SUMF1 protein (in the homodimer) and only about 10% of the SUMF2 protein (in the heterodimer) remained in the first washing of the immunoprecipitates (data not shown).

The interaction between SUMF1 and SUMF2 was confirmed by the detection of heterodimers between the endogenous proteins in Cos7 cells and in primary human fibroblasts. The endogenous SUMF1 was immunoprecipitated from total protein extracts with the anti-SUMF1 antibody, and western blotting of the immunoprecipitates with the anti-SUMF2 antibody showed more than one SUMF2-specific band, possibly corresponding to different glycosylated forms of the protein (Fig 2B, lane 1; supplementary Fig 3B online, lane 2). SUMF1 was also detected on the same filters on immunoblotting with the anti-SUMF1 antibody (Fig 2B, lane 4; supplementary Fig 3B online, lane 4). No specific bands were evident on immunoblotting with the pre-immunization sera (Fig 2B, lanes 2,3; supplementary Fig 3B online, lanes 1,3).

Altogether, these results show that both the endogenous and the overexpressed SUMF1 and SUMF2 proteins can form homodimers and heterodimers.

We also investigated whether SUMF2 was able to interact with the sulphatases with or without SUMF1. Cos7 cells were transfected with different combinations of tagged *SUMF1*, *SUMF2*, *IDS* and *SGSH* cDNAs (*SUMF1–HA* + *SUMF2–Flag* + *IDS–Myc*; *SUMF2–Flag* + *IDS–Myc*; *SUMF2–Myc* + *SGSH–Flag*; *SUMF1–Myc* + *SUMF2–HA* + *SGSH–Flag*; *SUMF1–Myc* + *SGSH–Flag*). Surprisingly, western blotting of the anti-Myc immunoprecipitates with the anti-Flag and anti-HA antibodies showed that SUMF2 was able to stably associate with IDS and with SGSH alone or in a complex with SUMF1 (Fig 2C). The loading control of this experiment is shown in the supplementary information online (supplementary Fig 4B online).

Overall, these results indicated that SUMF2 modulates the activity of SUMF1 through a direct interaction with SUMF1 and the sulphatases.

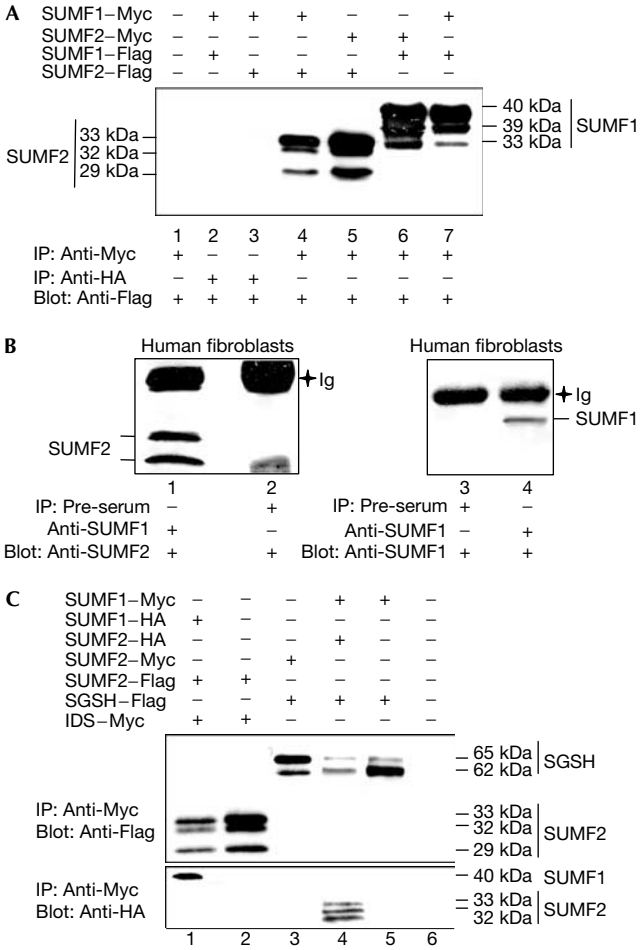

Fig 2 | SUMF1, SUMF2 and sulphatase interactions. (**A**) SUMF1 and SUMF2 form homodimers and heterodimers. Cos7 cells were co-transfected with the indicated plasmids. Immunoprecipitation (IP) and immunoblotting (Blot) were performed with the anti-Myc and anti-Flag antibodies. As controls, nontransfected and transfected cells were immunoprecipitated with the anti-Myc and anti-HA (haemagglutinin) antibodies, respectively. (**B**) Endogenous SUMF1 and SUMF2 heterodimerize. Co-immunoprecipitation was performed on fibroblast extracts, with the anti-SUMF1 and anti-SUMF2 antibodies. Pre-immunization serum was used as a control. (**C**) SUMF2 associates with IDS or SGSH with and without SUMF1. After Cos7 co-transfection, immunoblotting of the anti-Myc immunoprecipitates was performed with the anti-Flag and anti-HA antibodies.

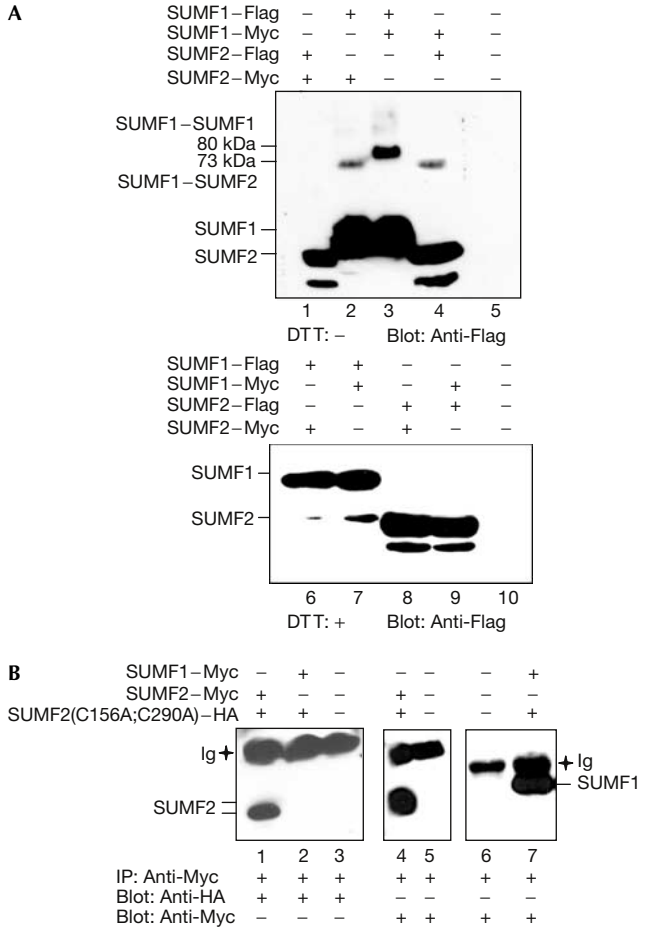

Fig 3 | Cysteine-mediated dimers. (**A**) SUMF1–SUMF1 homodimers and SUMF1–SUMF2 heterodimers are cysteine mediated. Cos7 cells were co-transfected with the indicated plasmids. The cells were treated with *N*-ethylmaleimide and analysed with nonreducing gradient SDS–polyacrylamide gel electrophoresis (SDS–PAGE) gels, or the extracts were treated with dithiothreitol (DTT) and analysed with reducing SDS–PAGE gels. The filter was detected with the anti-Flag antibody. (**B**) Cys 156 and Cys 290 of SUMF2 are essential for the SUMF1–SUMF2 and not for the SUMF2–SUMF2 interactions. Co-immunoprecipitation (IP) was performed with the anti-Myc and anti-HA antibodies.

Although the similarity between the SUMF1 and SUMF2 proteins is high, they differ in an important characteristic. Whereas SUMF1 has 11 cysteines, SUMF2 has only two throughout its entire length. To determine whether the interactions seen between these proteins are mediated by these cysteines, we loaded an 8–16% nonreducing gel with protein samples prepared from cells that had been transfected with different combinations of *SUMF1* and *SUMF2* tagged cDNAs (*SUMF2–Flag* + *SUMF2–Myc; SUMF1–Flag* + *SUMF2–Myc*; *SUMF1–Flag* + *SUMF1–Myc; SUMF1–Myc* + *SUMF2–Flag*). The cells were treated with the alkylating reagent *N*-ethylmaleimide (NEM), which acts on free thiols and prevents the reduction of existing disulphide bonds. On immunoblotting with the anti-Flag antibody, and together with the monomeric forms of SUMF1 and SUMF2, we detected further high-molecular-mass bands. The band at about 80 kDa corresponds to the SUMF1–SUMF1 homodimer (Fig 3A, lane 3), and that at 73 kDa to the SUMF1–SUMF2 heterodimer (Fig 3A, lanes 2,4). Although present, it was difficult to detect ~144-kDa band of the SUMF1–SUMF2 heterotetramer (data not shown), and we were unable to detect any band corresponding to the SUMF2–SUMF2 homodimer (Fig 3A, lane 1). This suggested that SUMF2 homodimerization is not mediated by intermolecular disulphide bridges. When the samples were treated with dithiothreitol (DTT), the high-molecular-mass bands disappeared, as they were converted to the monomeric forms (Fig 3A, lanes 6–10).

To confirm that SUMF1–SUMF2 heterodimerization (but not SUMF2–SUMF2 homodimerization) is mediated by the two SUMF2 cysteines (Cys 156 and Cys 290), we mutated these to alanines by changing the three bases of the two codons. Cos7 cells were co-transfected with either *SUMF1–Myc* + *SUMF2 (C156A;C290A)–HA* or *SUMF2–Myc* + *SUMF2(C156A;C290A)–HA* cDNAs. As expected, the co-immunoprecipitation showed that the mutated form of SUMF2 could no longer interact with SUMF1, although it was still capable of dimerizing with wild-type SUMF2 (Fig 3B, lanes 1,2). SUMF2–Myc and SUMF1–Myc were detected by western blotting with the anti-Myc antibody (Fig 3B, lanes 4,7). Altogether, these data show that the Cys 156 and Cys 290 residues of SUMF2 are important for the formation of the heterodimer, but not for SUMF2 homodimerization. The results also suggest that these cysteines might be crucial for the stability of the quaternary structures of SUMF1 and SUMF2.

What is the function of SUMF2 in the ER? We have previously shown that SUMF1 greatly enhances the activity of overexpressed sulphatases, which indicated that SUMF2 might also be capable of activating the sulphatases, although perhaps with different affinities/specificities as compared with SUMF1 (Cosma *et al*, 2003). To test this hypothesis, we transfected Cos7 cells with several sulphatase cDNAs (*ARSA*, *ARSC*, *ARSF*, *IDS*, *SGSH*, *GALNS*, *GNS*) and co-transfected each sulphatase with either *SUMF1* (as the controls) or *SUMF2* cDNAs. We did not observe any significant enhancing effects of SUMF2 on these sulphatase activities when tested at 24, 48 and 72 h after transfection (Fig 4). Therefore, SUMF2 is not able to induce the activation of the sulphatases, and it is likely that it has no FGly-generating activity.

Another possibility would be that SUMF2 modulates the enhancing effects that SUMF1 has on the sulphatase activities, possibly through a direct interaction with SUMF1 and/or with the sulphatases themselves. To test this hypothesis, we triple transfected Cos7 cells with sulphatase cDNAs (*SGSH*, *IDS*, *ARSB* or *ARSC*), with *SUMF1* cDNA and with increasing amounts of *SUMF2* cDNA. The enzymatic activities of the sulphatases were tested using the protein extracts. Surprisingly, we saw that the activity of the overexpressed sulphatases markedly decreased with increasing concentrations of SUMF2 (*SUMF2*:*SUMF1*:*IDS* DNA ratios of 1/3:1:1, 2/3:1:1 to 1:1:1), with respect to the activity of cells that were double transfected with *SUMF1* and the sulphatase cDNAs (Fig 5A, and data not shown). Western blotting confirmed the increasing amounts of SUMF2 and the constant amounts of SUMF1 and the sulphatases in the transfected samples (Fig 5A, and data not shown).

These decreased activities of the sulphatases on overexpression of SUMF2 suggested that a stoichiometric amount of SUMF1 and SUMF2 is crucial for the maximum enhancing effects of SUMF1 on the overexpressed sulphatases. We envisage that the

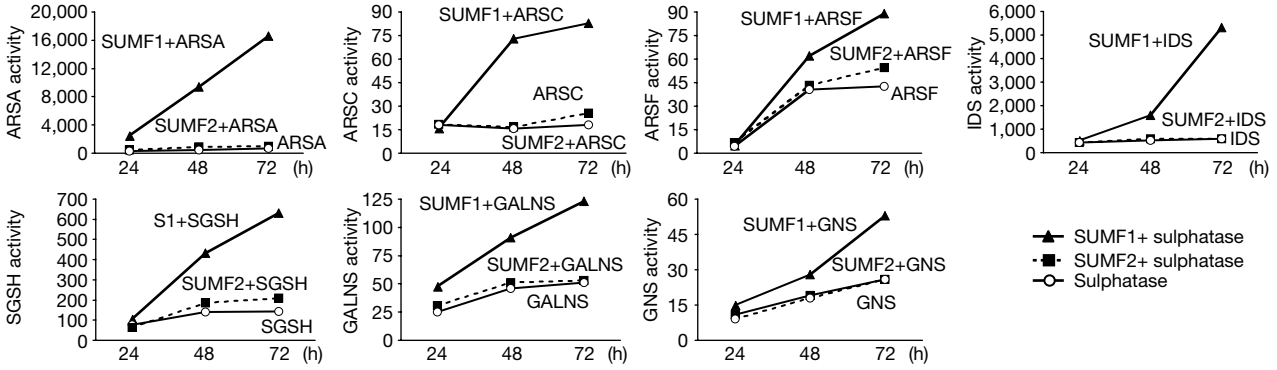

Fig 4 | Activity of SUMF2 on sulphatases. The *ARSA*, *ARSC*, *ARSF*, *IDS*, *SGSH*, *GALNS* and *GNS* complementary DNAs were transfected alone or in combination with the *SUMF1* or *SUMF2* cDNAs in Cos7 cells, as indicated. The enzymatic activities are expressed as nmol/mg protein.

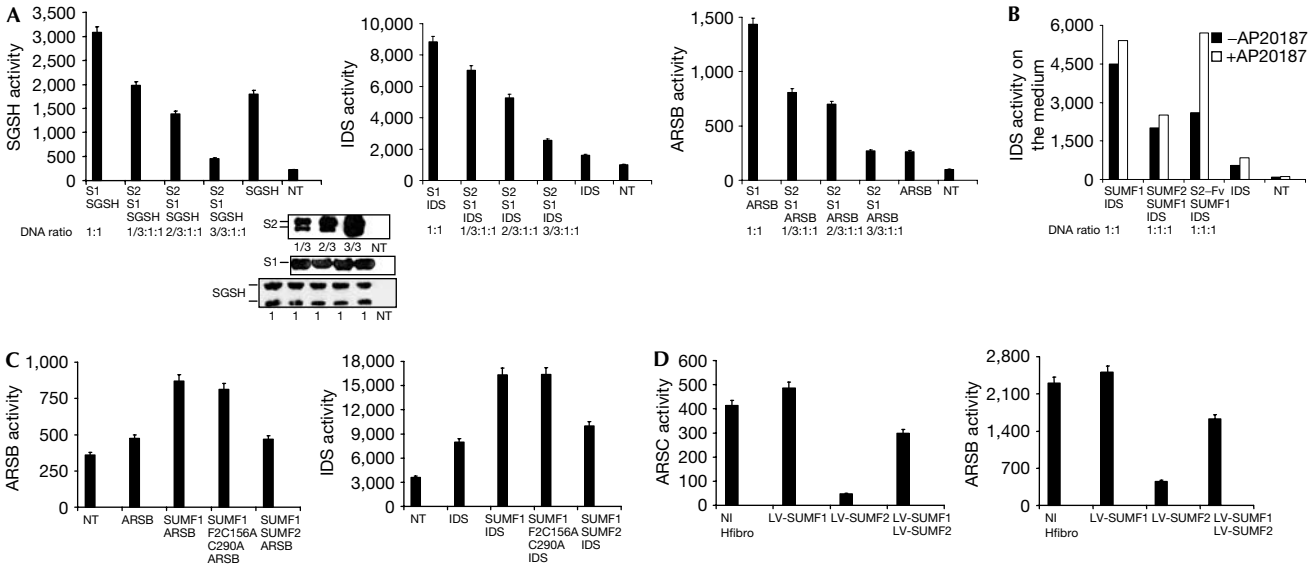

Fig 5 | SUMF2 inhibits SUMF1. (**A**) SUMF2 inhibits the enhancing activity of SUMF1 on the sulphatases. Cos7 cells were transfected with the *SGSH*, *IDS* and *ARSB* complementary DNAs alone or in combination with *SUMF1* (S1) or both *SUMF1* and *SUMF2* (S2) at different DNA ratios, as indicated. The enzymatic activities are shown. The amounts of SUMF1, SUMF2 and SGSH were detected by western blotting. (**B**) Cos7 cells were transfected with *IDS* cDNAs alone or in combination with *SUMF1* or both *SUMF1* and *SUMF2*–Fv or *SUMF2* at a 1:1:1 DNA ratio, as indicated. AP20187 was added at 24 h after transfection, for 24 h. The IDS enzymatic activities are shown. (**C**) C156A and C290A *SUMF2* mutations abolish the inhibitory activity of SUMF2 on SUMF1. Cos7 cells were transfected with the *ARSB* or *IDS* cDNAs, with the *ARSB* or *IDS* and *SUMF1* cDNAs, with the *ARSB* or *IDS*, *SUMF1* and *SUMF2* or *SUMF2(C156A;C290A)* cDNAs. The enzymatic activities of IDS and ARSB are shown. NT, nontransfected. (**D**) SUMF2 inhibits the activities of the endogenous sulphatases. Human fibroblasts were infected with the lentiviral vectors (LV) carrying either *SUMF1* or *SUMF2* cDNA, or double infected with both LV vectors. The endogenous ARSC and ARSB activities are shown. NI Hfibro, noninfected human fibroblasts.

stoichiometric equilibrium between SUMF1 monomers and SUMF1–SUMF2 homodimers and heterodimers regulates the amount of FGly generation within the sulphatases.

We also determined whether mutations in the two cysteines in SUMF2 would abolish the inhibitory activity of SUMF2 on SUMF1. Cos7 cells were triple transfected with sulphatase cDNAs (*ARSB* or *IDS*), *SUMF1* and the mutated form of *SUMF2* (*SUMF2(C156A;C290A)*) and analysed for the effects of the SUMF2 mutant on the enhancing effects of SUMF1. This showed that SUMF2(C156A;C290A) does not inhibit the enhancing effects of SUMF1 on the sulphatase activities (Fig 5C) and that to hamper SUMF1 activity, SUMF2 should, most likely, interact with SUMF1.

Finally, we determined whether SUMF2 can inhibit the enhancing effects of SUMF1 on the activities of the endogenous sulphatases in human fibroblasts. For this, we produced lentiviral vectors (LV) carrying the *SUMF1* and *SUMF2* cDNAs. Three different human fibroblast cell lines were infected with the same amount of viral particles of LV-*SUMF1* and LV-*SUMF2*, and coinfected with both LV-*SUMF1* and LV-*SUMF2*. In all three of the cell lines, the LV-*SUMF2* vector markedly decreased the activities of endogenous ARSC, IDS and ARSB, and this effect was rescued

by SUMF1 in the samples that were double infected with LV-*SUMF1* and LV-*SUMF2* (Fig 5D, and data not shown). Again, it is possible that high levels of SUMF2 in this cell type alter the ratios between the monomers and dimers to a point where the amount of active SUMF1 is limiting.

To dissociate SUMF2 from SUMF1, we forced the complete homodimerization of SUMF2 through the use of a homodimerizer system (ARIAD). Fv is a modified human FKBP12 protein that can be homodimerized by the synthetic drug AP20187 with a high efficiency. A chimeric *SUMF2–Fv–Flag* construct was generated and transfected in Cos7 cells. The protein was expressed and, as expected, it localized in the ER, as confirmed by the ERAB marker (supplementary Fig 2D online). We then triple transfected *SUMF2–Fv–Flag*, *SUMF1* and *IDS* cDNAs. As controls, we transfected the cDNAs of *IDS* alone, *IDS* and *SUMF1*, and *IDS* with *SUMF1* and *SUMF2*. At 24 h after addition of AP20187, SUMF2 was homodimerized and did not inhibit SUMF1 (Fig 5B). Furthermore, we have evidence that overexpression of *SUMF1* facilitates homodimer formation and that SUMF1 homodimers cannot activate the sulphatases, which suggests that SUMF1 is active as a free monomer (data not shown). By forcing the homodimerization of SUMF2–Fv, it is likely that we sequestered SUMF2 from the SUMF1/SUMF2–Fv heterodimer, leaving the free monomers of SUMF1 that are capable of activating IDS. Altogether, these data allow us to postulate that a balance exists in the cells between the SUMF1 and SUMF2 monomers, homodimers and heterodimers. Furthermore, and as shown above, SUMF2 can associate with the sulphatases with or without SUMF1. It is therefore possible that a stoichiometric equilibrium between the SUMF1–SUMF2–sulphatase complex and SUMF1 and SUMF2 homodimers is the regulatory mechanism that controls the amounts of free SUMF1, and thus ultimately the amounts of active sulphatases in different cells and tissues.

In conclusion, as SUMF2 colocalizes and interacts with SUMF1 and/or the sulphatases inhibiting SUMF1 function, it can be considered as a modulator of SUMF1 activity in vertebrates. Thus, ultimately, SUMF2 controls the amounts of the FGly-activated sulphatases. Determining whether this mechanism is sulphatase specific and/or tissue specific will be the object of future studies.

METHODS

Constructs, cell lines and lentivirus production. See the supplementary information online.

Northern blotting and real-time PCR. See the supplementary information online.

Transient transfection, western blotting and immunoprecipitation. Cos7 and 293T cells were transiently transfected with the polyfect Qiagen reagent, according to the manufacturer's instructions. The proteins were separated on a reducing SDS–polyacrylamide gel electrophoresis gel, or on a native SDS gel after NEM (10 mM) treatment of the cells. The immunoblots were detected with anti-SUMF1 (peptide 323–328, which exclusively recognizes SUMF1), anti-SUMF2 (full length), anti-Myc (Upstate, Waltham, MA, USA), anti-HA (Roche, Basel, Switzerland) and anti-Flag (Sigma, St Louis, MO, USA) antibodies. The immunoprecipitation was accomplished by mixing 1 mg of the protein extracts with the anti-Myc, anti-SUMF1 or anti-SUMF2 antibodies. The immunocomplexes were recovered with protein-A–Sepharose (Sigma).

Deglycosylation. The protein extracts from transfected cells were denaturated at 100 °C in glycoprotein denaturing buffer, and treated with Endo H (Biolabs, Hitchin, UK) and PNGase F (Biolabs) according to the manufacturer's instructions.

Sulphatase enzymatic analysis and immunofluorescence staining. See the supplementary information online.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

We thank G. Diez-Roux for her suggestions on the manuscript, A. Zullo for suggestions on the immunofluorescence experiments and V. Rivera (ARIAD) for providing the homodimerization kit (www.ariad.com/regulationkits). We are grateful for support from EMBO YIP, from the Telethon Foundation and from Transkaryotic Therapies Inc.

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