SS18 Together with Animal-Specific Factors Defines Human BAF-Type SWI/SNF Complexes

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Abstract

Background: Nucleosome translocation along DNA is catalyzed by eukaryotic SNF2-type ATPases. One class of SNF2-ATPases is distinguished by the presence of a C-terminal bromodomain and is conserved from yeast to man and plants. This class of SNF2 enzymes forms rather large protein complexes that are collectively called SWI/SNF complexes. They are involved in transcription and DNA repair. Two broad types of SWI/SNF complexes have been reported in the literature; PBAF and BAF. These are distinguished by the inclusion or not of polybromo and several ARID subunits. Here we investigated human SS18, a protein that is conserved in plants and animals. SS18 is a putative SWI/SNF subunit which has been implicated in the etiology of synovial sarcomas by virtue of being a target for oncogenic chromosomal translocations that underlie synovial sarcomas.

Methodology/Principal Findings: We pursued a proteomic approach whereby the SS18 open reading frame was fused to a tandem affinity purification tag and expressed in amenable human cells. The fusion permitted efficient and exclusive purification of so-called BAF-type SWI/SNF complexes which bear ARID1A/BAF250a or ARID1B/BAF250b subunits. This demonstrates that SS18 is a BAF subtype-specific SWI/SNF complex subunit. The same result was obtained when using the SS18-SSX1 oncogenic translocation product. Furthermore, SS18L1, DPF1, DPF2, DPF3, BRD9, BCL7A, BCL7B and BCL7C were identified. ‘Complex walking’ showed that they all co-purify with each other, defining human BAF-type complexes. By contrast, we demonstrate that human PHF10 is part of the PBAF complex, which harbors both ARID2/BAF200 and polybromo/BAF180 subunits, but not SS18 and nor the above BAF-specific subunits.

Conclusions/Significance: SWI/SNF complexes are found in most eukaryotes and in the course of evolution new SWI/SNF subunits appeared. SS18 is found in plants as well as animals. Our results suggest that in both protostome and deuterostome animals, a class of BAF-type SWI/SNF complexes will be found that harbor SS18 or its paralogs, along with ARID1, DPF and BCL7 paralogs. Those BAF complexes are proteomically distinct from the eukaryote-wide PBAF-type SWI/SNF complexes. Finally, our results suggest that the human bromodomain factors BRD7 and BRD9 associate with PBAF and BAF, respectively.

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Introduction

Gene expression programs determine cell identity and response to endocrine stimuli, as has been demonstrated most dramatically by the generation of induced pluripotent stem cells with the Oct4, Sox2, Klf4 and c-Myc transcription factors [1]. Such epigenetic programming involves many nucleosome remodeling activities [2]. Besides covalent nucleosome modifications such as acetylation and methylation, a second type of nucleosome remodelling involves translocation of nucleosomes along chromosomal DNA [3–5] as well as the catalysis of alternative nucleosome conformations, and even nucleosome eviction [6–8]. These nucleosome transactions are catalyzed by SNF2 type enzymes, a group of ATPases that belongs to the SFII ATPase superfamily that includes many helicases [9]. The present paper is concerned with a subtype of the nucleosome remodeling SNF2 enzymes that are uniquely characterized by a C-terminal bromodomain, represented in yeast by Snf2 and Sth1, in Drosophila by brahma and in humans by BRM and BRG1.

The C-terminal bromo domain-bearing SNF2 enzymes are found in so-called SWI/SNF multiprotein complexes and are conserved in most eukaryotes. They are implicated in transcriptional regulation and multiple DNA repair pathways [10–25]. These large multi-protein complexes consist of at least 4 evolutionarily conserved core subunits represented in man by SMARCB1 and the SMARCA2/A4, SMARCC1/C2 and SMARCD1/D2/D3 paralogs [26], and a large number of ancillary subunits, some of which define SWI/SNF complex subtypes. Interestingly, SWI/SNF complexes were identified as biochemical factors that dramatically reduce the amount of time required to reprogram mouse embryonic fibroblasts into iPSCs at the hand of recombinant transcription factors [27], underscoring the
importance of SWI/SNF in epigenetic programming processes [28]. Indeed, SWI/SNF has been mapped to some 50,000 human chromosomal sites in one cultured human cell line, demonstrating that this protein complex is a feature of many cis-acting regulatory elements, including DNA replication origins [29].

In mice and humans, at least 20 different SWI/SNF complex subunits have been reported (Table 1) [10,30–43]. ‘Core’ subunits are found in virtually all cellular SWI/SNF complexes, whilst others define SWI/SNF complex subtypes. There are two broad classes of SWI/SNF complexes known; BAF-type SWI/SNF complexes (BRG1/BRM-associated factors) bear either one of ARID1A/BAF250a or ARID1B/BAF250b, whilst PBAF (Polybromo-associated BAF) complexes harbor both ARID2/BAF200 and polybromo/BAF180 subunits [38,39,44,45]. Functionally, ARID1B/BAF250b was shown to be required to maintain ES cell identity [46] whilst ARID1A/BAF250a is required to permit proper ES cell differentiation with retinoic acid [47]. Furthermore, the SMARCC variant BAF170 is expressed less upon ES cell differentiation [43,48,49]. Similarly, the switch from one actin related subunit, BAF53A, to its paralog BAF53B appears to play a

Table 1. Abundance of purified proteins* in each TAP-tag preparation.

| Protein alternative names | polyA mRNA* | TAP | INI1 | SS18 | SS18SSX1 | BCL7A | BCL7C | DPF2 | BRD9 | PHF10 |
|--------------------------|-------------|-----|------|------|----------|-------|-------|------|------|-------|
| BRG1 SMARCA4             | 5.93        | 0   | 0.303| 5.337| 4.440    | 2.039 | 2.290 | 1.662| 0.141| 0.984 |
| BAF250A SMARCA2, ARID1A  | 1.45        | 0   | 0    | 1.339| 0.730    | 0.116 | 0.280 | 0.179| 0    | 0.028 |
| BAF250B ARID1B, OSA1     | 1.63        | 0.027| 5.692| 2.079| 0.379    | 1.766 | 1.485 | 0.027| 0.027|
| BAF200 ARID2, zipzap     | 2.04        | 0   | 0    | 2.728| 1.540    | 0.179 | 0.638 | 0.315| 0.028| 0     |
| BAF180 Polybromo-1       | 3.02        | 0   | 0.122| 0    | 0.029    | 0.029 | 0.122 | 0    | 0    | 0.884 |
| BAF170 SMARCC2           | 4.36        | 0   | 0.457| 0    | 0        | 0.248 | 0    | 0    | 1.769|
| BAF155 SMARCC1           | 9.25        | 0   | 1.532| 2.793| 2.360    | 0.438 | 1.432 | 1.154| 0.084| 1.745 |
| BAF60A SMARCD1           | 6.94        | 0   | 2.981| 5.813| 3.467    | 1.239 | 2.043 | 2.831| 0.080| 0.468 |
| BAF60B SMARCD2           | 4.01        | 0.15| 3.037| 3.037| 0.784    | 2.054 | 1.477 | 0.072| 0.630|
| BAF60C SMARCD3           | 7.69        | 0   | 0.719| 7.161| 4.080    | 1.762 | 2.875 | 2.384| 0    | 0.607 |
| BAF57 SMARCE1            | 3.02        | 0   | 0    | 1.102| 0.346    | 0    | 0.346 | 0.16 | 0    | 0     |
| BAF53A ACTL6A, ArpN8     | 9.97        | 0   | 0.957| 5.190| 1.154    | 3.642 | 3.217| 0.957| 1.61 |
| BAF53B ACTL6B, ArpNx     | 8.37        | 0.233| 0    | 6.305| 6.305    | 2.511 | 2.511| 0.874| 0.369| 0.52  |
| BAF47 SMARCB1, INI1, SNF5| 1.68        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0    | 0     |
| BAF45A PHF10             | 5.81        | 0   | 0.931| 5.449| 3.160    | 1.154 | 3.160| 0.551| 0.245| 0.823 |
| BAF45B DPF1              | 3.08        | 0   | 0.086| 0    | 0        | 0    | 0    | 0    | 0    | 0     |
| BAF45C DPF3, CERD4       | 0.50        | 0   | 0    | 0    | 0.110    | 0    | 0.110| 0    | 0    | 0     |
| BAF45D DPF2, REQ, UBID4  | 0.26        | 0   | 0    | 0    | 0        | 0    | 0.105| 0    | 0    | 0     |
| SS18 SYT, SSXT           | 2.80        | 0   | 0    | 4.623| 1.610    | 0.101| 1.371| 1.873| 0    | 0     |
| SS18L1 CREST             | 3.73        | 0   | 0    | 9.00 | 9.00     | 0.78 | 0    | 2.16 | 1.000| 0     |
| BCL7A nd                 | 9.92        | 0   | 0    | 0    | 0        | 0    | 0.585| 0.585| 0    | 0     |
| BCL7B Hom s 3            | 4.01        | 0   | 0    | 0    | 0.029    | 0    | 0    | 0    | 0    | 0     |
| BCL7C nd                 | 4.18        | 0   | 0    | 0    | 1.783    | 0    | 11.915| 0    | 0    | 0     |
| BRD7 CELTIX-1            | 7.69        | 0   | 0.646| 0    | 0        | 0    | 0    | 0    | 0.645|
| BRD9 MU-RMS-40.8         | 8.37        | 0   | 0    | 0.686| 0.186    | 0    | 0.089| 0    | 4.505| 0     |
| SSX1 nd                  | 3.73        | 0   | 0    | 1.154| 0        | 0    | 0    | 0    | 0     |
| GLTSCR1 GSCR1            | 1.20        | 0   | 0    | 0.066| 0        | 0    | 0    | 0    | 0.292|
| SRRM2 nd                 | 8.15        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| MYBBP1A p160             | 3.72        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| NONO NMT55, p54(nrb)     | 2.80        | 0   | 0    | 0.186| 0        | 0    | 0    | 0    | 0     |
| NUA1 nd                  | 3.23        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| SPFX PF5                 | 11.42       | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| DDX3X HLP2               | 12.18       | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| DDX17 p72                | 11.86       | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| RMM14 COAA               | 7.10        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| DDX5 p68                 | 3.73        | 0   | 0    | 0    | 0        | 0    | 0    | 0    | 0     |
| actin actg1              | 3.07        | 0   | 0    | 4.109| 1.371    | 4.109| 6.499| 2.481| 2.831| 0.778|

*Protein abundance is represented by the exponentially modified Protein Abundance Index [96].
*mRNA abundance was estimated from probe set fluorescence signal intensities, as recommended by Affymetrix (see Data S2).
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key role in neuron progenitor differentiation [41,50]. Tissue
specific expression of paralogous subunits has also been reported
for the BAF60 variants [32,31–33], as well as for the minor SWI/
SNF subunits DPF1 and D [41,42,50].

Strikingly, multiple SWI/SNF subunits function as tumor
suppressors in man and mouse, adding a key medical dimension
to SWI/SNF research [24,34–66]. For instance: the INI1
floxed mice is the most lethal tumor suppressor mouse model reported
to date [35], suggesting a decisive role for SWI/SNF in cell
proliferation control. Cell cycle roles for SWI/SNF-type complexes
have indeed been documented in human and in model
organisms [67–78]. Another link to cancer is provided by the SS18-SSX
onc fusion proteins [79]. Synovial sarcomas are aggressive soft-tissue tumors
accounting for about ten percent of all human soft-tissue sarcomas
[80]. Characteristic for synovial sarcomas is the t(X;18)(p11.2;q11.2)
translocation which is found in over 95% of all synovial sarcoma
cases and results in the fusion of the SS18 (also called Syt) gene on
chromosome 18 with one of the highly homologous SSX genes,
SSX1, SSX2 or SSX4, on the X chromosome and consequently the
expression of SS18-SSX fusion proteins [81–84]. These transloca-
tion events are believed to be the main molecular basis of this
disease [79]. Orthology of the SS18 protein also exists in plants.
They are positive regulator of cell proliferation in lateral organs,
such as leaves and flowers and appear to control aspects of cell
proliferation together with DNA sequence-specific GRF transcrip-
tion factors [85,86]. Mammalian SS18 has been reported to
associate with SWI/SNF chromatin remodeling complexes and to
interact with BRG1 and BRM proteins [87–90]. In order to identify
protein interactors of the SS18 and SS18-SSX proteins and to
characterize the SS18 and SS18-SSX complexes we exploited a
Tandem Affinity Purification (TAP) tagging approach combined
with mass spectrometric analysis [91].

We found SS18 to be present in BAF-class human SWI/SNF
chromatin remodeling complexes. Purification of SS18-SSX1
revealed that this onc fusion protein resides in the same
complexes. Interestingly, we detected several additional putative
SWI/SNF interactors [92–95]. Complex walking revealed the
presence of these proteins in the same BAF SWI/SNF complexes
as SS18, refining observations made by others [41,43]. Overall, we
conclude that human SS18 and its parologue SS18/1 CREST
together with; double PHD finger factors (DPF1,-2, -3), the B-cell
CLL/lymphoma 7 protein family members (BCL7A, -B, -C) and
BRD9 are specific to BAF-class SWI/SNF complexes, whilst
BRD7 and PHF10 characterize PBAF complexes. Furthermore,
with the exception of BRD7 and BRD9, quantitative mass
spectrometry analysis demonstrates that the major proteomic
interaction partners of all these factors are SWI/SNF subunits,
indicating that they are bona fide BAF-type SWI/SNF complex
subunits.

Results

TAP-tag purification of SWI/SNF complexes

In order to define the protein complexes harboring known and
suspected human SWI/SNF subunits we generated stable human
embryonic kidney cell (HeK293) clones transduced with retroviral
TAP-tag fusion expression constructs [91]. The following eight
TAP-fusions were purified and analyzed by mass spectrometric
analysis; INI1, SS18 and its oncogenic fusion product SS18-SSX1,
BCL7A and BCL7G, DPF2, PHF10 and BRD9 (Figure 1A). The
proteomic data we have collected (Table 1, Data S1) is
schematized in Figure 1B, where the thickness of the edges reflect
copurification efficiency [96].

INI1

INI1 is a core subunit of SWI/SNF complexes that is also
known as hSNF5, SMARCBD1 or BAF47. In our hands the yield of
SWI/SNF complexes obtained with INI1TAP has consistently been
comparatively low. For instance, in most INI1TAP preparations we
detect BRG1 but not BRM, and ARID1A but not ARID1B
(Table 1). This is consistent with BRG1 and ARID1A mRNA
levels being 2–3 times that of their respective paralogs in HeK293
cells (Table 1, Data S2), a fact that is also reflected in the yields of
these paralogous subunits in all the purifications (Table 1).
Another indication that the INI1TAP construct is not amenable to
very high yield SWI/SNF purifications is that of all the proteins we
employed here to purify SWI/SNF complexes, only two, PHF10
and BRD7, are detected by INI1TAP, whilst INI1 was detected in
all the reciprocal purifications (Table 1).

In keeping with a role as a core SWI/SNF subunit, INI1TAP
purifications harbored both PBAF and BAF-specific SWI/SNF
subunits (Table 1, Table 2). The comparatively higher yield of the
BAF-specific subunits ARID2 and polybromo versus the BAF-
specific ARID1A and ARID1B suggests that INI1-bearing BAF
complexes are more preponderant than INI1-bearing BAF
complexes in HeK293 cells, in line with the higher expression
level of the PBAF-specific polybromo subunit (Table 1).

SS18 and the oncogenic SS18-SSX fusions are BAF
subunits

SS18TAP purifications yielded high levels of SWI/SNF (Table 1,
Figure 1). All known core subunits were found, consistent with
previous work [87,97]. Since both ARID1A and ARID1B but no
ARID2 nor polybromo peptides were found, SS18 appears to be
specific to both the ARID1A and ARID1B-bearing BAF-class
variants of SWI/SNF (Table 1). Furthermore, several other potential
SS18 interactors were identified, including GLioma Tumor
Suppressor Candidate Region gene 1 protein (GLTSCR1), zinc
finger protein ubi-d4 (DPF2), B-cell CLL/lymphoma 7A (BCL7A)
and bromodomain containing protein 9 (BRD9) (Table 1, Figure 1B).

Because the chromosomal translocation t(X;18)(p11.2;q11.2)
results in production of the oncogenic SS18-SSX1 protein fusion it
was of interest to compare the proteomic environments of SS18
and the SS18 onc fusions. Essentially, purification of SS18-
SSX1TAP resulted in the same set of interactors as purification of
SS18TAP, with the exception of peptides originating from the
SSX1 moiety of the onc fusion protein (Table 1). All subunits of
the SWI/SNF BAF variant complex were identified, as well as the
novel interactors GLTSCR1, DPF2 and its paralog DPF1, BRD9,
and BCL7A and its paralogs BCL7B and C (Table 1, Figure 1B).
We conclude that, similarly to SS18, the SS18-SSX1 onc fusion
protein also resides in both the ARID1A and ARID1B-bearing
BAF variants of human SWI/SNF.

DPF2 resides in BAF

DPF2, also known as ubi-d4 or Requiem, is ubiquitously
expressed and implicated in apoptosis [92]. It belongs to the d4
family which in humans consists of three paralogous genes: neuro-
d4 (DPF1), ubi-d4 (DPF2) and cer-d4 (DPF3) [98,99]. This gene
family is not present in any of the currently sequenced plant
genomes. Figure 2A shows that DPF factors harbor a conserved N-
ter minal domain (Piam14051, [100]), a central C2H2-type
Kruppel zinc finger motif with potential nucleic acid binding
activity and C-terminal double paired finger PHD domains that
have been shown to mediate conditional protein-protein
interactions [42,101,102]. DPF1, 2, 3 and PHF10 were named BAF45A-
D [41] because they were found in biochemical SWI/SNF
preparations.
The DPF2TAP purification results indicate that DPF2 resides mainly in ARID1-bearing BAF complexes, since no polybromo or ARID2 peptides were identified, whilst high confidence ARID1A and ARID1B peptides were detected (Table 1). Furthermore, like SS18TAP, DPF2TAP co-purified BCL7A and BRD9 as well as the SS18 paralog, SS18l1. Association of DPF2 with SS18 was further confirmed by co-immunoprecipitation (Figure 2C).

PHF10 resides in PBAF

PHF10 harbors two PHD domains but it is not a member of the DPF paralog group as it lacks a central Krüppel zinc finger motif, and harbors a SAY domain that is conserved in animals but not plants [103] (Figure 2A). In contrast to DPF2TAP, PHF10TAP was second only to INI1TAP in its yield of the PBAF-specific subunits ARID2 and polybromo (Table 1), demonstrating a strong association with PBAF-class SWI/SNF complexes. None of the BAF-associated SS18, DPF or BCL7 factors were detected in PHF10TAP preparations, suggesting that PHF10 indeed resides in a distinct subset of SWI/SNF complexes. Complete exclusion of PHF10 from BAF complexes may not be the case however, since one high confidence ARID1A-derived peptide was identified. Whether this reflects physiological subunit exchange between subtypes of SWI/SNF complexes or mal-assembled complexes remains an open question.
Notably, high confidence BRD7 peptides were detected, similar to the INI1TAP purification (Table 1), suggesting that PHF10 forms PBAF-type SWI/SNF complexes that can harbor BRD7 but not BRD9 since PHF10TAP did not pull down BRD9, in contrast to SS18TAP, SS18-SSX1TAP and BCL7CTAP which did pull down BRD9 (Table 1, Figure 1B).

BCL7 proteins reside mainly in BAF
Similar to multiple SWI/SNF subunits, BCL7 family members have been implicated in carcinogenesis [93,94]. The presence of BCL7 family members in SWI/SNF complexes has been reported before [43]. We succeeded in purifying BCL7A TAP and BCL7CTAP-associated proteins (Table 1, Figure 1B). In both cases and in contrast to INI1TAP and PHF10TAP, next to the core SWI/SNF subunits we also recovered more BAF-specific ARID1A and ARID1B subunit peptides (Table 1, Figure 1B), suggesting that BCL7 factors are mainly subunits of BAF complexes. The comparatively low levels of the PBAF-specific subunits in the BCL7CTAP preparations may be due to the high levels of BCL7CTAP in the cell line that was employed (Figure 1A) and indicate that the distinction between BAF and PBAF complexes by BAF subunits can be blurred operationally (Figure 1B). The absence of the PBAF-specific SWI/SNF subunit PHF10 from both BCL7TAP purifications strengthens the notion that BCL7 factors mainly associate with the BAF variants of SWI/SNF, however. Furthermore, the fact that BCL7C TAP co-purified DPF2, like...
BCL7A\textsuperscript{TAP}, as well as the DPF2 paralogs DPF1 and DPF3 suggest that these two DPF2 paralogs also associate with BAF complexes. Indeed, co-purification of DPF1 and DPF3 parallels the purification results obtained with SS18-SSX1\textsuperscript{TAP} (see above) again pointing towards the BAF variants of SWI/SNF. Finally, like DPF2\textsuperscript{TAP}, BCL7C\textsuperscript{TAP} also pulled down SS18\textsuperscript{1TAP} (Table 1, Figure 1B), a paralog of SS18 also known as CREST which has previously been linked physically to ARID1B [104], again strengthening the conclusion that BCL7A and BCL7C are mainly subunits of the BAF variants of SWI/SNF complexes. Interestingly, orthologs of BCL7 can only be found in sequenced animal genomes.

BRD9 associates with BAF

A well established function of bromodomains is to recognize specific acetylated lysines. The paralogous catalytic subunits of SWI/SNF, BRG1 and BRM harbor one C-terminal bromodomain that is closely related to the six bromodomains of polybromo, but quite distinct from the bromodomains of BRD7 and BRD9 [105–107].

A FLAG-BRD7 fusion has been reported to purify PBAF complexes [43]. We were not able to successfully perform BRD7\textsuperscript{TAP} purifications (data not shown). However, BRD9\textsuperscript{TAP} did yield significant mass spectrometry results (Table 1, Figure 1). BRD9\textsuperscript{TAP} yielded peptide hits for at least one paralog of each core SWI/SNF subunit and, contrary to what was reported for BRD7 [43,100], the presence of high confidence ARID1A and ARID1B peptides indicates inclusion of BRD9 in BAF complexes. This notion is buttressed by the presence of SS18 and BCL7C amongst the proteins co-purifying with BRD9\textsuperscript{TAP} (Table 1, Figure 1, Data S1). Association of BRD9 with SS18\textsuperscript{TAP} was further confirmed by co-immunoprecipitation (Figure 2C).

We quantified our mass spectrometry data on the basis of the exponentially modified protein abundance index (emPAI, Table 1, Figure 1) and this revealed that BRD9\textsuperscript{TAP} did not efficiently purify SWI/SNF (Table 1), in keeping with our gel electrophoresis analysis (Figure 1A). The major factors we identified in our BRD9 preparation are the DEAD box ATP-dependent RNA helicases DDX3X, DDX5 and DDX17 and the RNA binding factor RBM14/COAA (Table 1). Since Emerson and co-workers reported substantially higher ATPase activity in their BRD7 preparations than predicted by BRG1/BRM content, it may perhaps be that BRD7 also co-purifies the DEAD box RNA helicases DDX3X, DDX5 and/or DDX17 [43,109,110]. Similarly, RBM14/COAA is a nuclear receptor co-activator [111]. Furthermore, RBM14/COAA has previously been reported to associate with SS18 in yeast two hybrid assays [112,113]. However, arguing against a direct interaction between SS18 and RBM14, we did not detect RBM14/COAA when SS18\textsuperscript{TAP} or SS18-SSX1\textsuperscript{TAP} associated factors were purified (Table 1, Figure 1B).

Putative BAF associated proteins

Crabtree and colleagues [49] published a list of putative novel BAF-associated proteins which we have monitored in this data set. Hence, we also detected GLTSCR1 in our SWI/SNF complex preparations (Table 1). GLTSCR1 is a candidate tumor suppressor gene for gliomas [114]. As we detected GLTSCR1 in four of five BAF purifications (Table 1), our results support the notion that GLTSCR1 is a BAF-associated factor, but this will need to be confirmed directly.

Of the other putative novel BAF-associated proteins, we could detect NONO and its binding partner SFPQ [115,116], however, at levels that were not much higher than in control purifications (Table 1). Thus, although our data do not exclude an interaction with SWI/SNF, more experimental evidence is needed on this front. Finally, the proposed putative BAF-associated factors NUMA1, SRRM2 and MYBBP1A [49] were not detected in any of our SWI/SNF purifications (Table 1), suggesting weak biochemical association with SWI/SNF in the ‘293’ human embryonic kidney cell line, if any.

Discussion

Paralogous human SWI/SNF subunits are known to be expressed in tissue and signal specific fashion, generating alternative SWI/SNF complex configurations that can cooperate
with transcription factor networks to coordinate cell proliferation and differentiation. Here, we focus on SWI/SNF subunits that are absent from yeast but conserved in animals and plants (SS18) or only in animals (DPF, BCL7, and PHF10) (Table 2).

Essentially there are two types of human SWI/SNF complexes [32,44]; those that harbor the polybromo/BAF180 and ARID2/BAF250 subunits (PBAF-class) and those that harbor either ARID1A/BAF250s or ARID1B/BAF250s (BAF-class) [117]. A similar bi-partition exists in Drosophila melanogaster except that there is only one ARID1 ortholog, namely OSA [118,119]. Similarly, the fly genome only encodes one ortholog of the mammalian SMARCC (CG18740/moiria) and of BRD7/9TAP did not efficiently pull in the reciprocal experiment, BRD9TAP purifications contained SS18 and deuterostome animals (Table 2), together define a novel class and DPF factors, which can be found in both protostome [125] and deuterostome animals (Table 2), respectively mark PBAF versus BAF-type SWI/SNF complexes in a mutually exclusive fashion.

**Methods**

**Constructs**

Tandem Affinity Purification (TAP) constructs were generated by PCR using the oligomers indicated in parentheses and cloned into the XhoI and EcoRI restriction sites in the retroviral expression vector pXZ, whereby the TAP-tag sequence was fused to the coding sequences at their N-terminus [91]. SS18 (isoform 2, cgtactGAATT-CATGGTGTGGTGGAGAAAT, tgtctCGATGTCAGGGTCGGGACTGTA, tgtctCGATGTCAGGGTGACTGTA) and BRD7 (cgtactGAATTCAAGGGTGATACCAAGTCTCTTGTAATTTCCATACT) were transduced with virus containing supernatant in two infectious rounds of 24 hours in the presence of 8 µg/ml puromycin and tested for recombinant protein expression. Transduction of SS18 and SS18-SSX TAPtag fusions in the syo-1 synovial sarcoma cell line [128] were not successful (data not shown). Since Hek293 cells expressed the paralogous cancer-related minor SWI/SNF subunits, such as the putative GLTSCR1 subunit [49], and these may also be present in our data sets (Data S1), which can be mined by interested investigators.

**Cell culture and stable cell lines**

Human Embryonic Kidney (HeK293, ATCC CRL-1573) and phoenix cells were grown in Dulbecco’s modified Eagles medium (Invitrogen) supplemented with 10% FCS, penicillin 100 µg/ml and streptomycin 100 U/ml (Invitrogen) at 37 °C in 5% CO₂. Retroviral stable cell lines were generated as previously described [91]. Briefly, phoenix amphotropic packaging cells were transduced with 20 µg retroviral plasmid pXZ-SS18, pXZ-SS18-SSX1, pXZ-SS18-SSX2, pXZ-DPF, pXZ-BRD7, pXZ-BCL7A, pXZ-BCL7C, pXZ-PHF10 or pXZ-IN1 after which Hek293 cells were transduced with virus containing superantigen in two infectious rounds of 24 hours in the presence of 8 µg/ml polybrene. Clones were selected with 1 µg/ml puromycin and tested for recombinant protein expression. Transduction of SS18 and SS18-SSX TAPtag fusions in the syo-1 synovial sarcoma cell line [126] were not successful (data not shown). Since HeK293 cells expressed the transduced clones and could be expanded as desired, we performed our study with this cell line.
expressing TAP-tagged proteins were incubated with IgG sepharose beads (Pharmacia). After TEV cleavage the TEV eluates were pre-cleared with protein A beads and used for immunoprecipitation with anti-MYC or anti TY1 epitope antibodies. Proteins were eluted from the beads by peptide elution, loaded on a SDS-PAGE gel and visualized by silver staining. The same protocol was employed for all the purifications reported here.

Co-immunoprecipitation

Co-immunoprecipitations were performed on TEV cleavage eluates obtained as described above, using antibodies directed against BRM (Abcam 15597), DPF2 (Aviva systems biology ARP33221_P050) or BRD9 (Aviva systems biology ARP34803_T200), under the same conditions as the anti-MYC or TY1 immunoprecipitations in the TAPtag purification protocol. The immunoprecipitated proteins were separated by SDS-PAGE, transferred onto nylon filters and probed with anti-MYC antibodies, which recognize the transduced SS18-SSX1 (Figure 2B) or SS18 (Figure 2C) proteins.

Mass spectrometry

The silver stained gel lanes were cut into small pieces. After reduction and alkylation the proteins were trypsin (Promega) digested and extracted from the gel using trifluoroacetic acid (TFA). Peptides were sequenced using a nano-high-pressure liquid chromatography Agilent 1100 nanoflow system connected online to a 7-Tesla linear quadrupole ion-trap Fourier transform (FT) mass spectrometer (Thermo Electron, Bremen, Germany) essentially as described previously [129]. MSquant software package (http://www.msqquant.sourceforge.net) was used to parse the raw files and for generation of peak lists. The mascot algorithm was used to identify the proteins [130]. Exponentially Modified Protein Abundance Index (emPAI) factors were calculated as described previously [96], using high confidence peptides (Data S1, Mascot score ≥20, delta mass ±5, error ≤5; 400–6000 Da).

Expression profiling

Expression profiling was performed on four Hek293 polyA mRNA samples by microarray analysis using Affymetrix human exon array 1.0 ST according to manufacturer instructions (Data S2).

Supporting Information

Data S1 Mass spectrometry results, including: accession numbers, short protein descriptions, peptide sequences, associated Mascot score, peptide delta score and absolute calibrated mass relative error.

(xls)

Data S2 Quadruplicate polyA mRNA expression profile of Hek293 cells determined with the Affymetrix human exon array 1.0 ST platform.

(xls)

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Author Contributions

Conceived and designed the experiments: CL HGS EM XW. Performed the experiments: EM XW PWJ VS. Analyzed the data: EM XW PWJ VS HGS CL. Contributed reagents/materials/analysis tools: EM XW PWJ VS. Wrote the paper: CL EM.

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16. Acknowledgments

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Author Contributions

Conceived and designed the experiments: CL HGS EM XW. Performed the experiments: EM XW PWJ VS. Analyzed the data: EM XW PWJ VS HGS CL. Contributed reagents/materials/analysis tools: EM XW PWJ VS. Wrote the paper: CL EM.
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