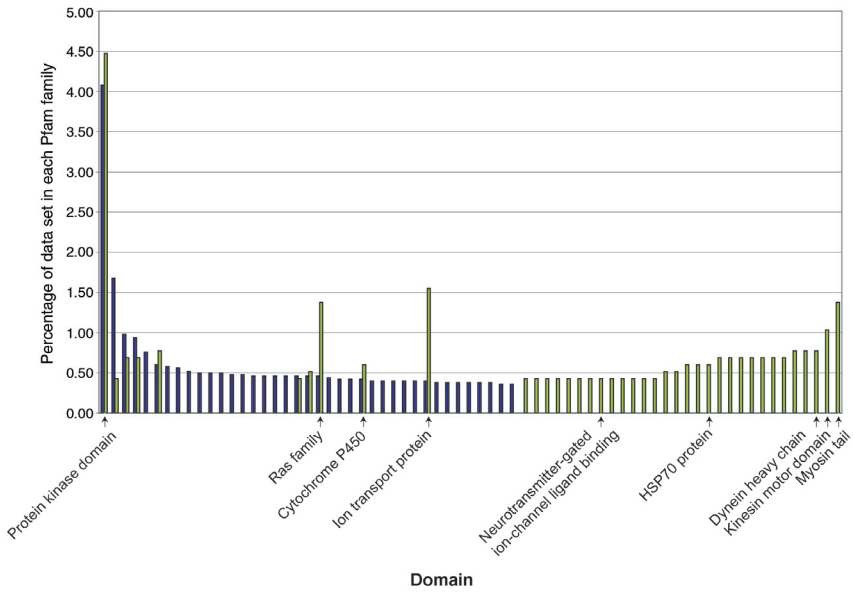

Figure 2 Domain analysis of the human ciliary proteome. We analyzed the domain distribution of 1,162 human ciliary proteins (green) in comparison with 5,000 random nonciliary proteins (blue) by querying the Pfam v11.0 database with RPS-BLAST with an *E* value threshold of 1×10^{-10}. The histogram depicts all protein families represented in at least 0.35% of either the ciliary or random datasets (*x* axis). Note the distinct representation of some domains in the ciliary proteome. See http://www.ciliaproteome.org/analyses.html to download a detailed image with all protein domains.

used the same methodology and similar general assumptions, reflecting primarily the experimental limitations of each protocol. By contrast, our combined proteome collection shows significant enrichment of *bona fide* ciliary and basal body proteins (90% and 83%, respectively). We also analyzed the extent of overlap among proteomics studies. Collectively, we found ~42% and 38% of ciliary proteins in the nonreciprocal (NR) and reciprocal (R) databases, respectively, to be reported in at least two independent studies (**Supplementary Fig. 1** online); the decreased overlap in the R versus NR databases is likely due to the choice of species used in the original proteomics investigation. Moreover, when compared in a pairwise fashion (**Supplementary Fig. 2** online), no two proteomics studies captured the same complement of ciliary proteins, further substantiating the benefit of an integrated resource. Therefore, despite the expected contamination of false positives in our database, our collection represents a highly saturated resource for proteins pertinent to the biology of the cilium.

Finally, we characterized the domain composition of the 1,162 human proteins in the ciliary proteome (**Fig. 2**). When we compared them with 5,000 random, nonciliary proteins, we found several domain families unique to the ciliary proteome, such as dynein heavy chain, kinesin motor domain, Hsp70 and gated ion channels, as well as several motifs enriched significantly, such as RAS and ion transport domains. We further specified this approach by assessing the domain distribution in proteins identified by mass spectrometry studies focused exclusively on cilia and flagella[1,7,10]. Although the overall distribution of domains was similar to that of the entire ciliary proteome, there were some significant differences. For example, we observed an absence of ion transport domains from the mass spectrometry ciliary subset (**Supplementary Fig. 3** online), raising the possibility that such proteins might be trafficked to the cilium under a specific stimulus and were thus not captured by the mass spectrometry studies. Intriguingly, we do not detect the enrichment of WD and tetracopeptide repeat (TPR) domains described previously[3], likely because of our significantly larger and more diverse data set.

The most daunting tasks ahead are to confirm experimentally and explore the functions of the proteins in the database. As the characterization of the cilium evolves, we are committed to appending experimental data such as protein localization images and RNA interference (RNAi) phenotypes. In addition, a dedicated link to each database entry permits the communication of errors and omissions; we welcome such input, since it will result in a robust representation of the ciliary proteome and a will be potent tool for the study of ciliary biology. We also invite colleagues to contribute biological information that will facilitate the further dissection of this organelle and the elucidation of its role in development and homeostasis.

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Note: Supplementary information is available on the Nature Genetics website.

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Testing for association between MeCP2 and the brahma-associated SWI/SNF chromatin-remodeling complex

To the Editor:

The methyl-CpG binding protein MeCP2 is encoded by an X-linked gene that is mutated in patients with the neurological disorder Rett Syndrome (RTT). Harikrishnan and colleagues report that MeCP2 functions through association with a brahma-containing SWI/SNF chromatin-remodeling complex[1]. Their conclusion is supported by biochemical evidence that MeCP2 coimmunoprecipitates and cofractionates with brahma (brm) plus other components of SWI/SNF in extracts of mouse NIH3T3 cells, and by immunocytochemical evidence that brahma and MeCP2 colocalize in NIH3T3 cell nuclei. Previous

studies, by contrast, claim that MeCP2 purified from rat brain lacks stably associated proteins[2], and SWI/SNF complexes purified by different methods are devoid of MeCP2 (ref. 3). In response to this discrepancy, we reinvestigated the relationship between MeCP2 and brm.

The coimmunoprecipitation experiments of Harikrishnan *et al.* lacked silver-staining analysis of the immunoprecipitated polypeptides and therefore did not exclude the possibility that contaminant proteins were isolated owing to antibody cross-reactivity. To address this issue, we immunoprecipitated mouse nuclear extracts using our own highly specific antibody to MeCP2 (hereafter the 'Hu' antibody; see **Supplementary Note** online) and relatively mild washing conditions (200 mM salt in our washing buffer compared with 500 mM in the buffer of Harikrishnan *et al.*). We prepared extracts from NIH3T3 cells and also from mouse brain, the primary functional tissue for MeCP2. In both cases, we isolated a single major polypeptide with an apparent molecular weight of 75 kDa (**Fig. 1a**,**b** and **Supplementary Fig. 1** online), which was confirmed as MeCP2 by mass spectrometry and immunoblotting (data not shown). Additional polypeptides that were present were also seen in the negative control lanes. We did not detect any specific coimmunoprecipitated proteins, in agreement with previous results[2]. In our hands, the antibodies used by Harikrishnan *et al.* (described in Jones *et al.*[4], or from Upstate and Abcam) did not immunopurify MeCP2, as visualized by silver staining after SDS-PAGE (data not shown), although the Jones antibody immunoprecipitated a trace of MeCP2 detectable by immunoblotting (**Fig. 1c**). We did not detect any SWI/SNF components by immunoblot assay, however, in immunoprecipitates by either the Jones antibody (**Fig. 1c**) or the Hu antibody (data not shown).

We found that the antibody to brm employed by Harikrishnan *et al.* for mouse studies (Santa Cruz Biotech, N-19, sc-6450) reacts poorly with mouse brm but is highly reactive to human brm (**Supplementary Fig. 2** online; see also manufacturer's website). Therefore, we used this antibody to immunopurify the brm-associated SWI/SNF complex from a human source: HeLa cells. The purified complex contained SWI/SNF components but no detectable MeCP2 (**Fig. 1d**,**e**). For comparison with the mouse analysis, we immunopurified MeCP2 from HeLa cells using the Hu antibody to MeCP2. We identified two major polypeptides of ~75 kDa (**Fig. 1d** and **Supplementary Fig. 3** online) as MeCP2 by mass spectrometry and immunoblotting (**Fig. 1e**). Again, we did not detect any SWI/SNF components in the immunopurified human MeCP2 (**Fig. 1e**).

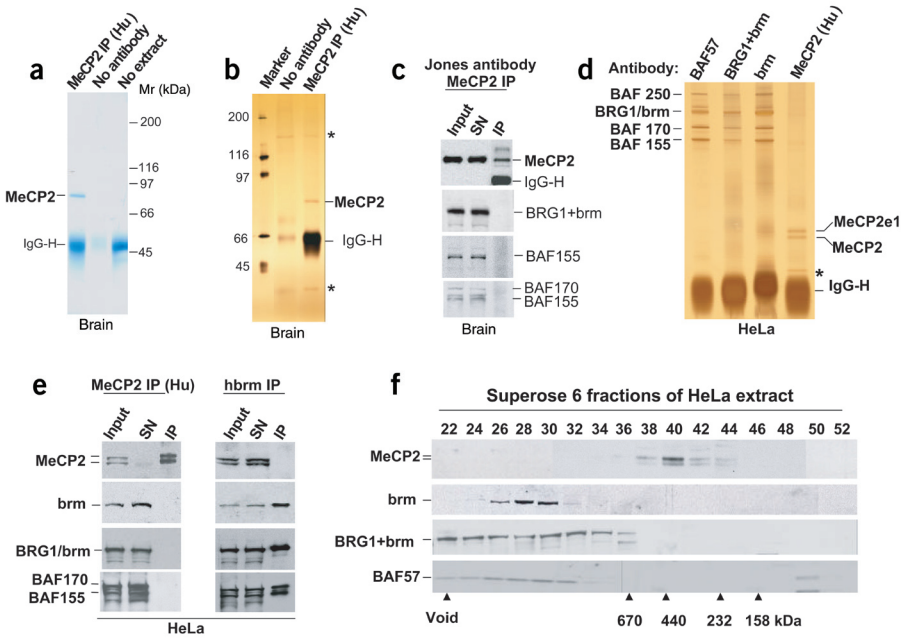

Figure 1 MeCP2 neither copurifies nor cofractionates with the SWI/SNF complex. (**a**,**b**) Coomassie- and silver-stained SDS gels showing MeCP2 immunopurified (IP) from mouse brain using our antibody ('Hu'). Control lanes show immunoprecipitation using protein A beads alone ('No antibody'), or using antibody beads without extract ('No extract'). Asterisks indicate contaminating polypeptides. The unusual gel mobility of MeCP2 is likely to be due to its abnormal amino acid composition: ~20% of the residues are positively charged. (**c**) Immunoblotting shows that MeCP2 immunoprecipitated from mouse brain contains no detectable SWI/SNF components (brm, BRG1, BAF 155 and BAF 170). The MeCP2 antibody in this IP was kindly provided by P. Jones[4] and was used in Harikrishnan's study. The nuclear extract (input), the supernatant after IP (SN), and the immunoprecipitated polypeptides (IP) are indicated. The Hu antibody was used in the immunoblotting analysis. (**d**) A silver-stained SDS gel shows proteins immunoprecipitated from HeLa nuclear extract with antibodies to MeCP2 and SWI/SNF components, including Brm (N-19), BRG1 and brm (J1), and BAF57. The two polypeptides of about 75 kDa were both identified as MeCP2 by mass spectrometry, which probably correspond to the two splicing isoforms of MeCP2. The polypeptide marked as MeCP2e1 contained the N-terminal peptide derived from the exon 1. (**e**) Immunoblotting shows that MeCP2 does not coimmunoprecipitate with brm and other SWI/SNF components in HeLa extracts. (**f**) Immunoblotting shows that MeCP2 in a HeLa nuclear extract fractionates separately from brm and other SWI/SNF components by Superose 6 gel-filtration chromatography. Fractions corresponding to marker proteins with known molecular weight are indicated at bottom.

Harikrishnan and colleagues claim that MeCP2 cofractionates with brm, BAF57 and INI1 by glycerol-gradient sedimentation. This result contrasts with previous findings that MeCP2 fractionates in the 400–500 kDa apparent molecular weight range[2], whereas SWI/SNF fractionates at over 1 MDa[3]. We carried out a fractionation experiment using high-resolution (FPLC) gel-filtration chromatography. The peak of MeCP2 was completely separated from the SWI/SNF components (**Fig. 1f**), arguing against stable association between these proteins. Low resolution may explain the failure of the glycerol gradients used by Harikrishnan *et al.* to fully separate MeCP2 and brm.

Harikrishnan *et al.* report colocalization of brm and MeCP2 in nuclei of NIH3T3 cells. This result differs from previous findings that MeCP2 is concentrated in condensed heterochromatin regions[5], whereas brm is enriched in active chromatin[6]. Differing subnuclear localizations of these proteins correlate with their opposing effects on transcription when tethered to promoters (MeCP2 represses transcription[7], whereas brm activates transcription[8]). We repeated the NIH3T3 cell experiment of Harikrishnan *et al.* and detected MeCP2 in heterochromatic DAPI bright spots, whereas brm was dispersed throughout the nucleus (**Fig. 2** and **Supplementary Fig. 4** online). We did not find any evidence for colocalization of these two proteins.

We cannot rule out that differences in cell extraction or other manipulations between

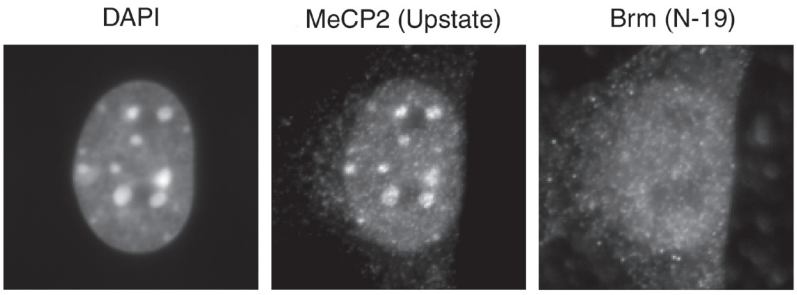

Figure 2 MeCP2 is concentrated in condensed heterochromatin regions, whereas brm is not. Indirect immunofluorescence analysis shows that MeCP2 is concentrated in condensed heterochromatin regions (as shown by bright DAPI-stained spots), whereas brm is dispersed throughout the nucleus. The antibodies used are shown in parentheses.

the two laboratories might contribute to the observed discrepancies (see **Supplementary Note** for further discussion). We note, however, that mice lacking brm[9] develop and grow normally, whereas mice without MeCP2 develop a severe neurological disorder that mimics RTT and die at ~10 weeks of age[10]. The absence of obvious RTT-like phenotypes in *brm*-null mice adds a genetic argument to a body of biochemical and cytological evidence questioning a major role for brm in mediating MeCP2 function.

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Harikrishnan *et al.* reply:

The arguments of Hu *et al.* are logically flawed and their findings inconsistent within their own work and in comparison with the work of others. Bird *et al.* originally published that HeLa cells lack endogenous MeCP2 (ref. 1), yet the authors purposefully draw on this cell type to support their current claims. We are critical of the suitability of this experimentation and therefore have reinvestigated these associations more closely. We repeated these experiments using four independent methods and five separate cell types to demonstrate clear and stable association between the SWI/SNF chromatin-remodeling complex containing Brahma with MeCP2 (ref. 2). The choice of HeLaS3 and other experiments by Hu *et al.* are unremarkable.

In contrast to Hu *et al.*, we found that immunoblotting with three MeCP2 antibodies (the polyclonal MeCP2 antibody produced by P.L. Jones, the polyclonal MeCP2 antibody produced by W. Wang and a monoclonal MeCP2 antibody (Mec-168; Sigma)) recognizes recombinant protein (**Fig. 1a**). Clearly, the antibody that we used in our previous study[2] specifically identifies human MeCP2; this is further confirmed by short interfering RNA (siRNA)[2] analysis, demonstrating not only the specificity of knockdown but also recognition of MeCP2 using alternative antibodies (**Supplementary Fig. 1** online). We examined endogenous MeCP2 by protein blot analysis and found that it was expressed in HeLa and HeLaS3 cells (**Fig. 1b**). The use by Hu *et al.* of this cell type to support their hypothesis is self-contradictory[1]. Next, we clearly found that immunopurified MeCP2 and BAF57 are associated with Brm, BAF155 and Sin3A in NIH3T3 and CEM-CCRF cells (**Fig. 1c**), consistent with the existence of a MeCP2-associated SWI/SNF complex[2]. In contrast, we did not detect an association in HeLaS3 cells (**Fig. 1c**).

Hu *et al.* claim MeCP2 should not associate with larger protein complexes[3], in contrast with results indicating the corepressor exists as a large >1 MDa complex[4]. Even more puzzling is the oversimplification that MeCP2 should not fractionate >0.5 MDa. Therefore, we prepared nuclear extracts[5] from CEM-CCRF, NIH3T3 and HeLaS3 cells and repeated gradient experiments that Hu *et al.* failed to test (**Fig. 1d**). MeCP2 cofractionates with Brm in CEM-CCRF ($n = 3$ independent experiments) and NIH3T3 ($n = 5$) cells but not in HeLaS3 cells ($n = 5$). Correspondingly, mammalian Sin3A-HDAC2 coeluted in fractions 9–14 with MeCP2 and Brm in CEM-CCRF and NIH3T3 cells. Unsurprisingly, the corepressor complex sedimented in fractions 11–18 in HeLaS3 cells (**Supplementary Note** online). We analyzed NF-κB/p50, which does not interact with Brm, and found that p50 did not cofractionate, indicating that gradient sedimentation allows reproducible separation of non-interacting polypeptides from high–molecular weight multisubunit complexes. Additionally, coimmunoprecipitation indicated specific interaction of MeCP2 with Brm from peak fractions (**Fig. 1e–g**). Careful analyses point toward consistency[2]: Brm coimmunoprecipitates with MeCP2 in CEM-CCRF and NIH3T3 fractions but not in HeLaS3. Hu *et al.* speculate incongruity of gradient resolution without presenting direct evidence to validate their views, when, in fact, there is an altered interaction.

Hu *et al.* conclude that MeCP2 does not exist with Brm by preparative chromatography, which is inconsistent with our experimental findings[2] and at odds with recent evidence[4]. Again, they challenge without providing sufficient experimental evidence to assess the same cell types. We thoroughly investigated this association by isocratic fractionation of nuclear extracts prepared from CEM-CCRF (**Fig. 2a**), NIH3T3 (**Fig. 2b**) and HeLaS3 cells (**Fig. 2c**). Furthermore, we examined cells in G_0 arrest, HeLa cells, rat