Development 125, 3955-3966 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV5234

The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes

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Accepted 23 July; published on WWW 14 September 1998

SUMMARY

The trithorax group gene *brahma* (*brm*) encodes an activator of *Drosophila* homeotic genes that functions as the ATPase subunit of a large protein complex. To determine if BRM physically interacts with other trithorax group proteins, we purified the BRM complex from *Drosophila* embryos and analyzed its subunit composition. The BRM complex contains at least seven major polypeptides. Surprisingly, the majority of the subunits of the BRM complex are not encoded by trithorax group genes. Furthermore, a screen for enhancers of a dominant-negative *brm* mutation identified only one trithorax group gene, *moira* (*mor*), that appears to be essential for *brm* function in vivo. Four of the subunits of the BRM complex are related to subunits of the yeast chromatin remodeling complexes SWI/SNF and RSC. The BRM complex is even more highly related to the human BRG1 and hBRM complexes, but lacks the subunit heterogeneity characteristic of these complexes. We present biochemical evidence for the existence of two additional complexes containing trithorax group proteins: a 2 MDa ASH1 complex and a 500 kDa ASH2 complex. These findings suggest that BRM plays a role in chromatin remodeling that is distinct from the function of most other trithorax group proteins.

Key words: Chromatin, Transcription, Homeotic, SWI/SNF, ATPase, Trithorax group

INTRODUCTION

The homeotic genes of the Antennapedia and Bithorax complexes (ANT-C and BX-C) encode transcription factors that specify the identities of body segments in *Drosophila* (Duncan, 1987; Kaufman et al., 1990). The transcription of these genes must be precisely regulated since their misexpression causes alterations in segment identity. The initial boundaries of homeotic gene transcription are established in the early embryo by transcription factors encoded by segmentation genes. During subsequent development, the restricted patterns of homeotic gene transcription are maintained by two globally expressed groups of regulatory proteins: the Polycomb group of repressors (Pc-G) and the trithorax group of activators (trx-G) (reviewed in Kennison, 1995; Pirrotta, 1997; Simon, 1995). Mutations in *Polycomb* (*Pc*) and other Pc-G genes cause homeotic transformations due to the ectopic transcription of multiple ANT-C and BX-C genes. Conversely, mutations in *trithorax* and other trx-G genes cause homeotic transformations due to the failure to maintain expression of homeotic genes. By maintaining states of homeotic gene expression established earlier in development, Pc-G and trx-G genes play critical roles in the control of cell fate.

Although the mechanism of action of most Pc and trx-G genes is not well understood, some of these proteins appear to regulate transcription by altering chromatin structure. This possibility was first suggested by the discovery that the PC protein contains a short domain – the chromodomain – which is conserved in a component of *Drosophila* heterochromatin, the HP1 protein (Paro and Hogness, 1991). Based on this similarity, it has been proposed that PC packages inactive homeotic genes into inaccessible complexes in the early embryo, thus preventing their subsequent transcription. PC and several other Pc-G proteins, including Polyhomeotic (PH), Posterior sex combs (PSC), Sex combs on midleg (SCM), Polycomblike (PCL), Enhancer of zeste (E(Z)), Suppressor of zeste-2 (SU(Z)2), and Additional sex combs (ASX), colocalize at a large number of sites on polytene chromosomes (Sinclair et al., 1998 and references therein; Peterson et al., 1997; Platero et al., 1996). Biochemical studies have demonstrated physical associations among PC, PH, PSC and SCM, and between two other Pc-G proteins, E(z) and Extra sex combs (ESC) (Franke et al., 1992; Jones et al., 1998; Kyba and Brock, 1998; Peterson et al., 1997; Strutt and Paro, 1997). These observations indicate PC physically interacts with other Pc-G proteins to repress transcription.

Genetic studies have suggested that trx-G proteins may also physically interact to regulate the transcription of homeotic genes. For example, mutations in many trx-G genes cause similar homeotic phenotypes, enhance mutations in other trx-G genes and suppress homeotic phenotypes produced by *Pc* mutations. Additional evidence suggesting that a complex of trx-G proteins regulates the transcription of homeotic genes has come from studies of a trx-G gene, *brahma* (*brm*). *brm* was identified in a screen for extragenic suppressors of *Pc* mutations (Kennison and Tamkun, 1988) and subsequently shown to be highly related to a yeast protein, SWI2/SNF2 (Tamkun et al., 1992), which functions as the ATPase subunit of a 2 MDa chromatin remodeling complex, the SWI/SNF complex (reviewed in Peterson and Tamkun, 1995).

What does the similarity of BRM to SWI2/SNF2 suggest about the mechanism of action of the BRM protein? Mutations in SWI2/SNF2 and other SWI/SNF subunits alter chromatin structure in vivo and can be suppressed by mutations in nucleosomal histones (Hirschhorn et al., 1992; Prelich and Winston, 1993). Purified SWI/SNF facilitates the binding of transcriptional activators to nucleosomal DNA in vitro by causing ATP-dependent alterations in nucleosome structure (Côté et al., 1994; Owen-Hughes et al., 1996). The SWI/SNF complex thus uses the energy of ATP hydrolysis to counteract the repressive effects of chromatin on transcription. These findings, together with the discovery that BRM is the ATPase subunit of a 2 MDa complex (Dingwall et al., 1995), suggested that a complex of trx-G proteins might counteract the repressive effects of PC or other components of chromatin on the transcription of ANT-C and BX-C genes.

It has recently become apparent that BRM and SWI2/SNF2 are members of a family of ATPases involved in chromatin remodeling (Pazin and Kadonaga, 1997; Peterson, 1996; Tsukiyama and Wu, 1997). For example, the yeast STH1 protein, which is highly related to both SWI2/SNF2 and BRM, is the ATPase subunit of a chromatin remodeling complex known as RSC (Remodels the Structure of Chromatin) (Cairns et al., 1996b). Although the RSC and SWI/SNF complexes are distinct in both size and subunit composition, four subunits of RSC are related to subunits of the SWI/SNF complex (Cairns et al., 1996b). Other differences between the two complexes suggest that they have distinct functions in vivo. For example, RSC is abundant and essential while the SWI/SNF complex is significantly less abundant and not essential for viability.

ATP-utilizing chromatin remodeling factors related to *brm* have also been identified in vertebrates, including the human BRG1 and hBRM proteins (Khavari et al., 1993; Muchardt and Yaniv, 1993). BRG1 and hBRM are the catalytic subunits of multiple large complexes with in vitro activities similar to those reported for the SWI/SNF and RSC complexes (Muchardt et al., 1995; Wang et al., 1996a,b). These human complexes contain polypeptides related to the four conserved subunits of the yeast SWI/SNF and RSC complexes as well as subunits that are unique to higher eukaryotes. A great deal of heterogeneity is present in the human complexes as many of the conserved subunits are encoded by gene families (Wang et al., 1996b). The result is a diversified array of related chromatin remodeling complexes whose in vivo roles remain to be determined.

Our understanding of the role of BRM and other trx-G proteins in transcriptional regulation has been limited by a lack of information concerning the subunit composition of the BRM complex. Does BRM interact with other trx-G proteins to activate the transcription of homeotic genes? In addition to BRM, only one subunit of the 2 MDa BRM complex has been identified to date, the SNR1 protein (Dingwall et al., 1995). SNR1 is related to both the SNF5 subunit of SWI/SNF and the SFH1 subunit of RSC. SNR1 is also highly related to the human INI1 protein, a subunit of both the BRG1 and hBRM complexes. Since genetic studies of chromatin remodeling factors in yeast have the potential to clarify the mechanism of action of BRM, we wanted to determine if the BRM complex is the *Drosophila* counterpart of either SWI/SNF or RSC. To address these issues, we purified the BRM complex from embryos and identified its subunits by peptide microsequencing. In a complementary approach to identify proteins required for BRM function, we screened for mutations and deficiencies that enhance a dominant-negative *brm* mutation and identified a non-overlapping set of genes. Finally, we present biochemical evidence for the existence of three distinct protein complexes containing trx-G gene products.

MATERIALS AND METHODS

Electrophoresis and western blotting

SDS-PAGE and western blotting were performed as described previously (Tsukiyama et al., 1995). The mouse monoclonal antibody 12CA5 (BabCo) was used to recognize the influenza hemagglutinin protein (HA) epitope of BRM-HA-6HIS. Affinity-purified rabbit polyclonal antibodies were used to detect ASH1 (Tripoulas et al., 1996), ASH2 (Adamson and Shearn, 1996) and BRM (Elfring et al., 1998). Rat antiserum was used to detect SNR1 (Dingwall et al., 1995). Affinity-purified, HRP-conjugated goat antibodies against rabbit, rat or mouse IgG (BioRad), and the Pierce SuperSignal or SuperSignal ULTRA chemiluminescence reagents were used to detect primary antibodies.

Purification of BRM and associated proteins from *Drosophila* embryos

Native protein extracts were prepared from 0-12 hour *w P[w$^+$,brm-HA-6HIS]92C; brm^2/Df(3L)th102, h ri Sb ca^2* embryos as described previously and stored at −80°C (Elfring et al., 1998). Extracts were thawed and PMSF was added to 100 μg/ml. Following centrifugation (10,000 ***g***, 10 minutes, 4°C), the supernatant was passed over a Sephadex G-25 Medium column (Pharmacia) equilibrated with 200 mM NaCl in Q buffer (25 mM sodium phosphate, pH 7.8, 10% glycerol, 0.1% Tween-20 and protease inhibitors [1 μg/ml each aprotinin, leupeptin, chymostatin and Pepstatin A]). G-25 eluate containing approximately 300 mg of protein was applied to a 10 ml Q-Sepharose HP column (Pharmacia) at a flow rate of 1 ml/minute and washed with 5 volumes 200 mM NaCl in Q buffer at 2 ml/minute. Bound proteins were eluted with a gradient (0.2 to 0.4 M NaCl in Q buffer) and the fractions containing BRM protein were identified by western blotting. Following the addition of fresh PMSF to 100 μg/ml and adjustment to 50 mM sodium phosphate (pH 7.8) and 425 mM NaCl, the BRM-containing fractions were loaded on a Ni-NTA Agarose (Qiagen) column equilibrated in binding buffer (50 mM sodium phosphate pH 7.8, 420 mM NaCl, 10% glycerol, 0.1% Tween-20, 100 μg/ml PMSF and protease inhibitors). 10 mg of protein were loaded per ml resin and the flow through was reapplied to the column. The column was washed with 14 volumes 8 mM imidazole in wash buffer (50 mM sodium phosphate adjusted to pH 6.0 with NaOH, 300 mM NaCl, 10% glycerol, 0.1% Tween-20, 100 μg/ml PMSF and protease inhibitors) followed by 4 volumes 30 mM imidazole in wash

buffer. Bound proteins were eluted with 2.5 volumes 125 mM imidazole in wash buffer. The fractions containing BRM protein were concentrated using a Centriprep-30 ultrafiltration unit (Amicon) and fractionated on a Superose 6 HR 10/30 column equilibrated in 50 mM sodium phosphate pH 7.8, 400 mM NaCl, 10% glycerol, 1 mM $MgCl_2$, 0.1 mM EGTA, 0.05% Tween-20 and protease inhibitors. 0.5 ml fractions were collected and those containing BRM protein were identified by western blotting and stored at −80°C.

Protein sequencing

Purified BRM complex was subjected to preparative SDS-PAGE on a 7% gel. Following brief Coomassie staining, gel slices containing each polypeptide band were excised, rinsed twice with 50% acetonitrile, frozen and shipped to the Harvard Microchemistry Facility (Cambridge, MA) for in-gel reduction, S-carboxyamidomethylation and digestion with trypsin (Promega). Peptide sequence information was obtained by microcapillary reverse-phase chromatography (Nash et al., 1996) coupled to the electrospray ionization source of a quadrupole ion trap mass spectrometer (Finnegan LCQ, San Jose, CA). For BAP74, BAP55, BAP60 and BAP47, the trypsin-digested material was separated by microbore high performance liquid chromatography using a Zorbax C18 1.0×150 mm reverse-phase column on a Hewlett-Packard 1090 HPLC/1040 diode array detector and individual peptides were subjected to Edman degradation on a Perkin Elmer/Applied Biosystems Procise 494A or 477A protein sequencer (Foster City, CA). Strategies for peak selection, reverse-phase separation and Edman microsequencing are as previously described (Lane et al., 1991). The following sequences were obtained: BAP155 LNPTEYLTSTACR and IPYIYIRPEIEK, BAP111 TEFIDEYEAEKLEYEK and (LI)NEIFSEAVVPDVR, BAP74 KFDDAAVQSDMK and LVTHFVQEFK, BAP60 AGVPGVPGVPGVPGPSLLQ and DLVPESQAYMDLLTFER, BAP55 KFYVDTNYVTVPR and VIDYAYANVIQSEPEY, BAP47 QEYDESGPSIVHR and GYSFTTTAER, and BAP45 AVSVNTSDTPVPR.

Analysis of BAP cDNA clones

EST cDNA clones derived from a 0-22 hour *iso1* cDNA library (Berkeley *Drosophila* Genome Project/HHMI EST Project, unpublished) were identified in BLAST searches of the *Drosophila* EST database and obtained from Genome Systems Inc. (St Louis, MO). Restriction fragments from cDNAs corresponding to BAP155 (LD15755 and LD12602) and BAP60 (LD12580 and LD09078) were subcloned and sequenced using the Applied Biosystems dRhodamine terminator cycle sequencing kit and an ABI310 automated sequencer. All ambiguous regions were sequenced on both strands. Genbank accession numbers for cDNA sequences are as follows: BAP155, AF071502 and BAP60, AF071503. LD13023 corresponds to BAP111 and LD19276 corresponds to BAP55. Sequence alignments were performed using the BLAST and MAP algorithms (Altschul et al., 1990; Gish and States, 1993; Huang, 1994).

The cytological position of each BAP gene was determined by hybridization of ^{32}P-labeled cDNA fragments to a high-density filter of *Drosophila* P1 genomic clones (Genome Systems Inc.). The following restriction fragments served as probes: LD12580 and LD19276, *Eco*RI-*Xho*I approximately 1.4 kb; LD12602 and LD15755, *Xho*I nucleotides 2542-3892 of the LD15755 cDNA sequence; and LD13023, *Bam*HI approximately 1.4 kb. Probes (25 ng in 30 ml hybridization buffer) were hybridized to the P1 filter using standard techniques, resulting in the identification of at least one P1 clone for each BAP. The cytological position of each P1 clone mapped by the Berkeley *Drosophila* Genome Project was confirmed by in situ hybridization to salivary gland polytene chromosomes using digoxigenin-labeled probes (Elfring et al., 1994). DNA probes were as above for LD12580, LD19276 and LD13023. A 1.3 kb *Xho*I-*Bam*HI fragment of LD12602 was used to confirm the position of BAP155.

Determination of copy number by Southern blotting

2.5 μg of *iso1* genomic DNA was digested with restriction enzymes, fractionated on 0.8% agarose gels and analyzed by Southern blotting as in Elfring et al. (1994). ^{32}P-labeled DNA probes corresponding to each BAP were prepared using the cDNA fragments described above. A cDNA fragment corresponding to nucleotides 2572-3494 of the *brm* RNA (Tamkun et al., 1992) was used as a positive control. Following hybridization, unbound probe was removed by extensive washing under low-stringency conditions (2× SSC, 0.1%SDS, 50°C).

Determination of the native molecular mass of trithorax group proteins

Native protein extracts were prepared as above from *w P[w$^+$,brm-HA-6HIS]92C; brm^2/Df(3L)th102, h ri Sb ca^2* embryos (for BRM and ASH1) or *w P[w$^+$,ISWI-HA-6HIS]19-4/TM6,Sb* embryos (for ASH2). 2 mg of protein were fractionated on a Superose 6 HR 10/30 FPLC column as described in Elfring et al. (1998). Column fractions were assayed for the ASH1, ASH2 and BRM proteins by western blotting as described above.

Coimmunoprecipitation assays

20 μl 12CA5 ascites fluid was incubated for 1 hour at room temperature with approximately 100 μl of Protein A-Affi prep beads (Bio-Rad, Richmond, CA) and 80 μl of incubation buffer (10 mM Hepes, pH 8.0, 1 mM EDTA, 10% glycerol, 50 mM NaCl) and antibodies were crosslinked to the beads using DMP (Harlow and Lane, 1988). 25 μl of the resulting immunoaffinity resin was added to 250 μg of embryo extract in a final volume of 125 μl of IP buffer (10 mM Hepes, pH 8.0, 100 mM NaCl, 10% glycerol, 0.05% Tween-20, 100 μg/ml PMSF and protease inhibitors). Embryo extracts were prepared as above from BRM-HA-6HIS embryos (*w P[w$^+$,brm-HA-6HIS]92C; brm^2/Df(3L)th102, h ri Sb ca^2*) or from control embryos (*y, Df(1)w67c2* for all but the ASH1 experiment, which used *Canton S*). Incubation was at 4°C with rocking for 3-16 hours. Following washing with 60 bead volumes of IP buffer, bound material was eluted by boiling for 5 minutes in Electrophoresis Sample Buffer (1% SDS, 5% glycerol, 40 mM Tris, pH 6.8, 1 mM EDTA, 50 mM DTT). Unbound and eluted proteins were analyzed by SDS-PAGE and western blotting (see above).

Drosophila stocks and genetic crosses

Flies were raised on a cornmeal-molasses-yeast-agar medium containing Tegosept and propionic acid at 25°C. Mutations and chromosome aberrations are described in Lindsley and Zimm (1992) or FlyBase (http://flybase.bio.indiana.edu) except as follows: *TrlR^{85}* (Farkas et al., 1994); *snr1^{R3}* (Dingwall et al., 1995); *Df(3L)v65c* (Budnik and White, 1987); *ash1^{22}* (Tripoulas et al., 1994) and *ash2^1* (Adamson and Shearn, 1996). The deficiency kits and many of the other stocks used in this study were obtained from the Bloomington *Drosophila* Stock Center. The generation of *P[w$^+$, brm^{K804R}]* is described in Elfring et al. (1998). *P[w$^+$, brm^{K804R}]22D* is an insertion of this transgene on the X chromosome which fails to rescue the recessive lethality of *brm*.

To identify dominant enhancers of *brm^{K804R}, y Df(1)w67c2 P[w$^+$, brm^{K804R}]22D* virgin females were crossed to males bearing a mutation or deficiency in *trans* to a balancer chromosome. The progeny were scored to identify mutations and deficiencies that cause male-specific lethality in the presence of the *P[w$^+$, brm^{K804R}]22D* transgene.

RESULTS

Purification of the BRM complex

To identify proteins associated with BRM, we purified the BRM complex from *Drosophila* embryos. To facilitate

purification, we used a *Drosophila* strain expressing BRM protein bearing a six histidine tag and HA epitope at its C terminus; this tag does not affect either the function of the BRM protein or its incorporation into the BRM complex (Dingwall et al., 1995; Elfring et al., 1998). Purification of BRM and associated proteins from transgenic embryo extracts was monitored by western blotting using polyclonal antibodies against the BRM protein. BRM represents approximately 0.1% of the protein in these extracts, as determined by quantitative western blotting (data not shown). The BRM protein was purified approximately 1,000 fold with a 4% yield using three chromatographic steps: Q-Sepharose anion exchange, Ni^{2+}-chelate affinity and Superose 6 gel filtration chromatography (Fig. 1A). Using this purification scheme, we consistently observed eight major proteins ranging from 195 to 45 kDa in size (Fig. 1B). Western blotting revealed that the 195 kDa protein in these preparations is BRM (data not shown).

The proteins that consistently copurify with BRM have been designated BRM-associated proteins (BAPs) and are referred to by their molecular mass in kDa (BAP45, BAP47, BAP55, BAP60, BAP74, BAP111 and BAP155). The same set of proteins copurify with BRM using an alternate purification protocol (Q-Sepharose, Superose 6 and Mono S cation exchange chromatography; data not shown). Thus, two different purification schemes identify the same set of seven polypeptides associated with BRM. Western blotting identified BAP45 as SNR1 (data not shown). To identify the other BAPs, microgram quantities of each polypeptide were resolved by SDS-polyacrylamide gel electrophoresis and sent to the Harvard Microchemistry Facility for peptide microsequence analysis by mass spectrometry and Edman degradation. A single peptide confirmed the identity of BAP45 as SNR1. For all other BAPs, two peptides were sufficient for unambiguous identification of *Drosophila* genes or EST sequences encoding the BAPs.

The BRM complex contains the four subunits conserved in the yeast and human SWI/SNF-like chromatin remodeling complexes

Peptides obtained from BAP155 matched translated *Drosophila* EST sequences allowing us to obtain and sequence a full-length cDNA encoding the BAP155 protein. The BAP155 protein is highly related to the BRG1/hBRM associated factors (BAFs) BAF155 and BAF170 (Wang et al., 1996b), and the yeast SWI3 (Peterson and Herskowitz, 1992) and RSC8 (Cairns et al., 1996b) proteins (Fig. 2; Table 1). Common to all of these proteins are three domains of unknown function: regions I, II and III (Fig. 2). In addition, the 440 residues between the N terminus of BAP155 and domain I are highly conserved in the human BAF155 and BAF170 proteins (39 and 34% identity, respectively), but not in the yeast SWI3 and RSC8 proteins (Fig. 2). SWI3 and RSC8 also lack the proline-rich domains immediately C-terminal to domain III that are present in BAP155 and its human counterparts.

Peptides obtained from BAP60 also matched translated *Drosophila* EST sequences allowing us to obtain and sequence a full-length cDNA encoding the BAP60 protein. The BAP60 protein is highly related throughout its length to BAF60a, BAF60b and BAF60c (Wang et al., 1996b), the human homologs of the yeast SWP73 and RSC6 proteins (Cairns et al., 1996a,b) (Fig. 3; Table 1). BAP60 is most closely related to BAF60a (72% identity), which is consistent with the characterization of BAF60c as a potentially tissue-specific subunit and with the identification of BAF60b as a component of a variant 500 kDa complex in mammals (Wang et al., 1996b). BAP60 is equally related to both yeast SWP73 and RSC6 proteins (approximately 16-28% identity); however, the yeast proteins contain two relatively large insertions within the approximately 370 amino acid segment conserved in their *Drosophila* and human relatives (Fig. 3). Thus the BRM complex contains four subunits (BRM, BAP155, BAP60 and BAP45/SNR1) that are conserved in the human BRG1 and hBRM complexes and in both the yeast SWI/SNF and RSC complexes (Table 1).

The BRM complex contains an HMG domain protein unique to higher eukaryotes

In addition to counterparts of the yeast SWI/SNF and RSC subunits, the BRM complex contains a polypeptide unique to

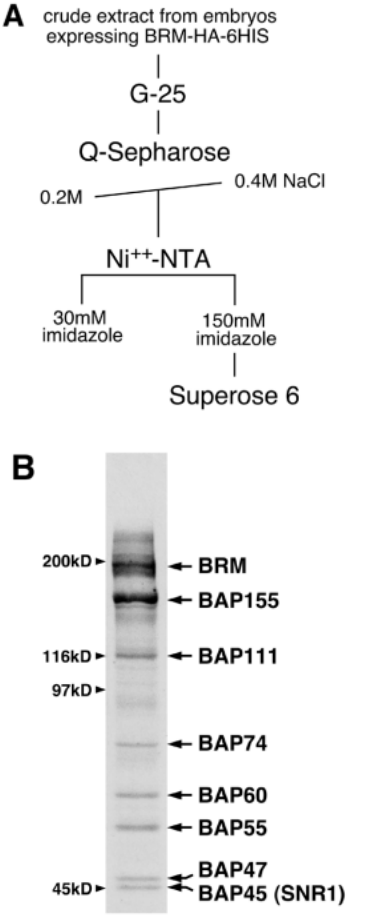

Fig. 1. Purification of the BRM complex from embryo extracts. (A) Summary of methods used to purify the BRM complex from *Drosophila* embryos. BRM-HA-6HIS is the epitope-tagged BRM protein. (B) BRM complex purified by this protocol was resolved on a 7% SDS-polyacrylamide gel and stained with Sypro-Orange. The seven abundant proteins that consistently copurify with BRM (BAPs) are designated by molecular mass and indicated by arrows. The positions of molecular mass markers are indicated by arrowheads. No polypeptides smaller than 45 kDa were detected on higher percentage gels using Sypro Orange, Coomassie blue, or silver staining methods (data not shown).

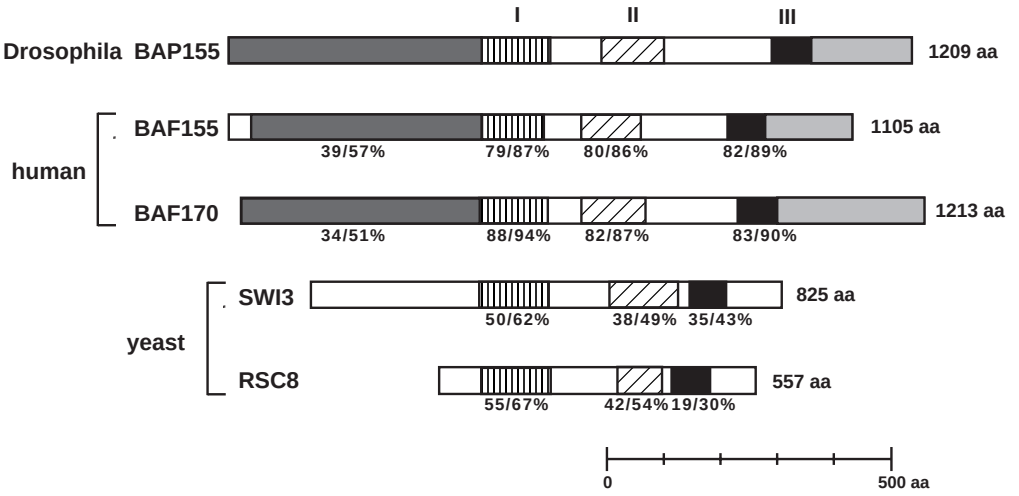

Fig. 2. *Drosophila* BAP155 is related to components of the yeast SWI/SNF and RSC, and human BRG and hBRM complexes. The full-length BAP155 protein is compared to the human BAF155 and BAF170 and yeast SWI3 and RSC8 proteins. Domains I, II and III (indicated by vertically striped, diagonally striped and black boxes, respectively) are highly conserved among these five proteins. Additional regions of similarity shared between BAP155 and its human relatives are indicated in dark gray and light gray. Conserved regions are as defined by Wang et al. (1996b). The % identity/% similarity to BAP155 is indicated below each conserved region. The C-terminal region (light gray) is a proline-rich region. Alignments were performed using the BLAST and MAP algorithms (Altschul et al., 1990; Gish and States, 1993; Huang, 1994).

higher eukaryotes. Peptide sequences obtained for BAP111 matched the translation of a *Drosophila* EST, LD13023, which encodes an HMG domain protein. This EST overlaps another *Drosophila* EST (LD03794) that was previously identified by Wang et al. (1998) in a search for sequences related to an HMG domain-containing subunit of the human BRG1 and hBRM complexes, BAF57. Like BAF57, the *Drosophila* BAP111 protein contains the conserved proline, tyrosine and lysine residues characteristic of HMG-domain proteins that recognize structured DNA without sequence specificity (Wang et al., 1998). The BAP111 subunit of the BRM complex is thus conserved in higher eukaryotes but is absent from the yeast SWI/SNF and RSC complexes (Table 1).

Two peptides identified BAP74 as the HSP70 cognate HSC4 (the product of the *Hsc70-4* gene). HSC4 is a constitutive (non-heat inducible) chaperone protein. Peptide sequences from BAP47 matched conserved regions of the non-muscle actins ACT1 and ACT2 (products of the *Act42A* and *Act5C* genes). Due to the extreme abundance of actin and HSC4 in the embryo, immunoprecipitation experiments were unable to demonstrate a clear association of these proteins with the BRM complex (data not shown). Consistent with our findings, both actin and an actin-related protein have recently been identified as subunits of the human hBRM and BRG1 complexes (K. Zhao, W. Wang, O. Rando, Y. Xue and G. Crabtree, personal communication).

Other proteins associated with BRM

Identification of the remaining three BAPs revealed proteins not previously reported to be subunits of chromatin remodeling complexes. Peptides from BAP55 matched the translation of a *Drosophila* EST that appears to encode a novel actin-related protein. Actin related proteins (Arps) are a functionally diverse group of proteins that share 17-64% sequence identity with actin (Frankel et al., 1994; Poch and Winsor, 1997). The translation of sequence obtained from both ends of the BAP55 cDNA revealed 38% identity with actin over a total of 239 amino acid residues (comprising the 157 N-terminal and 84 C-terminal residues of BAP55) suggesting it is one of the more divergent Arps. These regions of BAP55 are even less related to other known Arps. Because antibodies to BAP55 do not exist, we could not determine whether BAP55 is a nuclear protein and a bona fide subunit of the BRM complex by immunoprecipitation. However, it is intriguing that some of the most divergent Arps identified to date are nuclear proteins with reported roles in transcription and chromatin structure (Frankel et al., 1997; Jiang and Stillman, 1996).

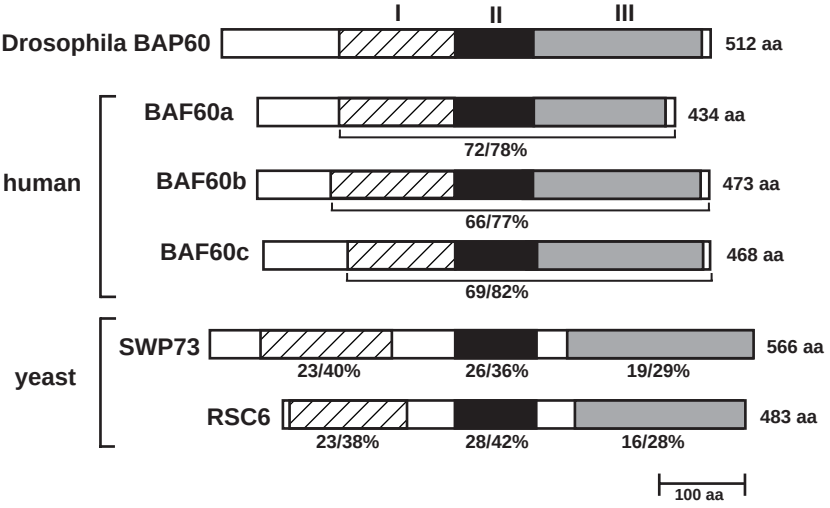

Fig. 3. *Drosophila* BAP60 is related to components of the yeast SWI/SNF and RSC, and vertebrate BRG and hBRM complexes. The full-length BAP60 protein is compared to the human BAF60a, BAF60b and BAF60c and yeast SWP73 and RSC6 proteins. These proteins are related throughout their lengths with the exception of divergent N termini and insertions in the yeast proteins separating blocks of homology I, II and III depicted by diagonal stripes or black or gray shading respectively. The % identity/% similarity to BAP60 is indicated below each protein. Alignments were done using the MAP algorithm (Huang, 1994). Domain boundaries for BAP60 are as follows: region I, amino acids 124-179; region II, 182-243; region III, 329-418.

Table 1. The relationship of the BRM complex to yeast and human SWI/SNF-like complexes

BRM complex subunit	RSC counterpart	SWI/SNF counterpart	BRG1/hBRM counterpart
BRM	STH1	SWI2/SNF2	BRG1, hBRM
BAP155	RSC8	SWI3	BAF155, BAF170
BAP111	none	none	BAF57
BAP74	?	?	?
BAP60	RSC6	SWP73	BAF60a, b, c
BAP55	?	?	?
BAP47	?	?	?
BAP45/SNR1	SFH1	SNF5	INI1

Polypeptides identified in the BRM complex are listed and their counterparts in the yeast SWI/SNF and RSC, and human hBRM and BRG1 complexes shown. "None" indicates the absence of a related sequence in the *S. cerevisiae* genome while a question mark indicates that related proteins exist but have not been reported as components of the indicated complexes.

The yeast SWI/SNF complex has been reported to associate with the RNA polymerase II holoenzyme (Wilson et al., 1996). This claim has been challenged (Cairns et al., 1996b) and conflicting reports have emerged regarding the mammalian hBRM and BRG1 complexes and PolII (Neish et al., 1998; Wang et al., 1996b). None of the seven BAPs are PolII subunits and antibodies against the second largest subunit of PolII failed to detect any antigen in purified BRM complex by western blotting (data not shown). Therefore PolII of *Drosophila* does not appear to be stably associated with the BRM protein in *Drosophila* embryo extracts.

The subunits of the BRM complex are encoded by single copy genes

Multiple genes related to BRM, BAP155, BAP60 and BAP45/SNR1 are present in yeast and humans (Table 1). To determine whether similar heterogeneity might exist in *Drosophila*, we searched for additional genomic sequences related to BAP genes by Southern blotting. After hybridization and washing under low-stringency conditions, all major bands detected were consistent with restriction maps of the BAP cDNAs; no crosshybridizing bands were detected for BAP155, BAP60, BAP55 or SNR1 (Fig. 4). By contrast, a *brm* genomic DNA fragment crosshybridizes under these conditions to ISWI, a divergent ATPase related to BRM (Fig. 4; see Discussion). Several additional weak signals were observed with the BAP111 probe. The BAP111 probe spans the HMG domain and may be detecting other HMG-Box genes. None of these weak signals persist after washing at high stringency (0.1× SSC, 0.1% SDS, 65°C). The *Drosophila* genome thus does not appear to contain multiple genes that could give rise to the subunit heterogeneity reported for the yeast and human counterparts of the BRM complex.

The BRM complex is one of three complexes containing trx-G proteins

None of the BAPs that we identified are known trx-G proteins. Since many of the trx-G genes have not yet been cloned, we asked whether they might encode any of the newly identified subunits of the BRM complex. Using a combination of hybridization to a filter containing mapped P1 clones (9216 clones with an average of 83 kb of genomic DNA per clone) and in situ hybridization to polytene chromosomes, we observed a single map location for each of the previously unmapped BAPs. The P1 clone number and cytological position for each of these BAPs is as follows: BAP155, P1# DS08140, map location 88E9-F2; BAP111, P1# DS00459, map location 8C9-13; BAP60, P1# DS03747, map location 11D5-10; and BAP55, P1# DS01093, map location 54A2-B. The P1 clone hybridizing to BAP155 is reported to map to 88E9-F2, very close to the location assigned to the trx-G gene *moira* (*mor*) (Kennison and Tamkun, 1988; Brizuela and Kennison, 1997; Lindsley and Zimm, 1992). None of the other BAPs map near known trx-G genes (Fig. 7). Thus, with the possible exception of *mor,* the sequence and chromosomal map location of the BAPs do not correspond to previously identified trx-G genes. We therefore conclude that the majority of trx-G proteins are not prominent subunits of the BRM complex.

We next examined whether other trx-G proteins might be substoichiometric components of the BRM complex. Based on their genetic properties, three of the best candidates for trx-G members that physically interact with BRM are ABSENT, SMALL or HOMEOTIC DISCS 1 and 2 (ASH1 and ASH2), and TRX (see discussion). Affinity-purified polyclonal antibodies against ASH1 detect three prominent bands in embryo extracts, the largest of which is 270 kDa (Fig. 5A). The predicted size of the ASH1 protein (244 kDa) and the variability in amount of the smaller bands detected in different experiments argues that the 270 kDa band represents full-length ASH1 and

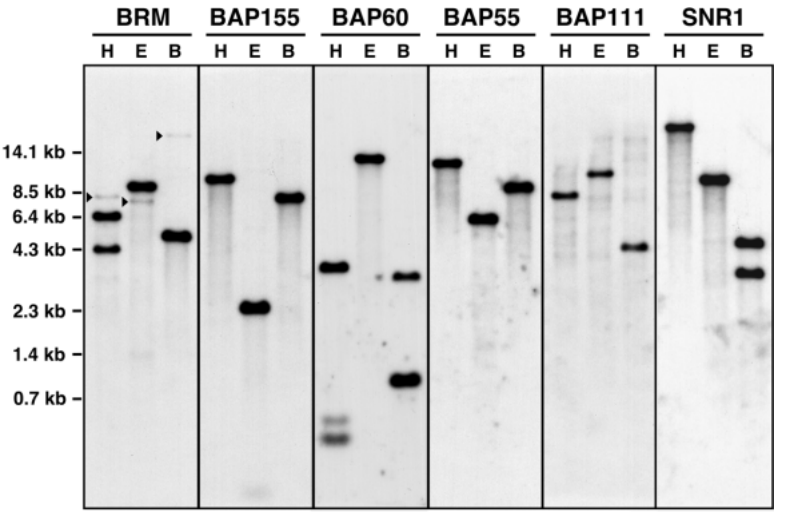

Fig. 4. *Drosophila* BAPs are encoded by single copy genes. Southern blots of *Drosophila* genomic DNA digested with *Hin*dIII (H), *Eco*R1 (E) or *Bam*H1 (B) were hybridized to BAP cDNA fragments or a *brm* genomic DNA fragment. After washing under conditions of low stringency (50°C, 2× SSC, 0.1%SDS) all the signals observed for BAP155, BAP60, BAP55, BAP111 and SNR1 corresponded to those predicted by the restriction maps of the BAP cDNAs and the SNR1 gene. Using the same hybridization and washing conditions, a *brm* genomic DNA fragment crosshybridizes to ISWI (bands indicated by arrowheads), which encodes an ATPase only 41% identical to BRM within the region covered by the probe. The weaker signals detected with the BRM and BAP111 probes disappeared upon high-stringency washing (65°C, 0.1× SSC, 0.1%SDS).

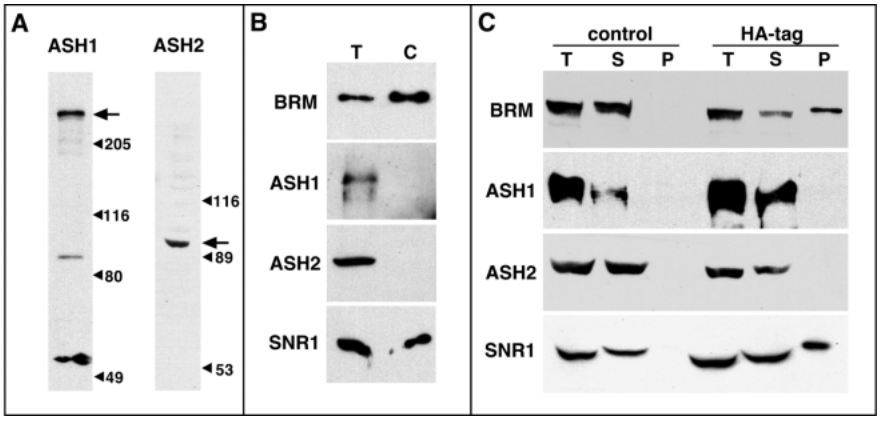

Fig. 5. ASH1 and ASH2 are not associated with the BRM complex in embryos. (A) Characterization of antibodies against ASH1 and ASH2. Western blots of *Drosophila* embryo extracts were probed with antibodies against ASH1 or ASH2. Polyclonal antisera against ASH1 and ASH2 detect prominent bands of 270 kDa and 94 kDa respectively (large arrows). The positions of molecular mass markers are marked by small arrowheads adjacent to their molecular mass in kDa. (B) ASH1 and ASH2 do not copurify with BRM. Western blots containing whole embryo extract (T) or purified BRM complex (C) were probed with antibodies against BRM, ASH1, ASH2 or SNR1. Note that equivalent BRM and SNR1 signals were observed in whole-embryo extract and purified complex, but no ASH1 or ASH2 was detected in the purified BRM complex. (C) Neither ASH1 nor ASH2 coimmunoprecipitate with the BRM protein. Immunoprecipitation was performed using an anti-HA monoclonal antibody and extracts made from wild-type embryos (control) or *P[w*$^{+}$*,brm-HA-6HIS]92C* transgenic embryos (HA-tag). Western blotting was performed on total extract (T), supernatant (S) and pellet (P) using the antibodies indicated at left. Note that SNR1 coimmunoprecipitates with BRM while ASH1 and ASH2 do not.

that the smaller bands are degradation products. Affinity-purified antibodies against ASH2 detect a single band of 94 kDa (Fig. 5A). Although the BRM, BAP45/SNR1, ASH1 and ASH2 proteins are readily detected by western blotting in whole embryo extracts, neither the ASH1 nor ASH2 proteins were detected in purified BRM complex (Fig. 5B). Similar experiments using antibodies against TRX did not yield reproducible results, presumably due to the low abundance and instability of this >350 kDa protein (data not shown).

We next examined whether ASH1 or ASH2 are physically associated with BRM in embryo extracts using a coimmunoprecipitation assay. BRM was immunoprecipitated using a monoclonal antibody directed against the HA epitope of BRM-HA-6HIS. The proteins present in the starting extract, the immunoprecipitated material, and the supernatant were assayed for BRM, BAP45/SNR1, ASH1 and ASH2 by western blotting. Identical immunoprecipitations using extracts prepared from embryos that do not express HA-tagged BRM protein served as a negative control. As expected, the BAP45/SNR1 protein coimmunoprecipitated with BRM from extracts prepared from transgenic embryos, but not control embryos (Fig. 5C). By contrast, neither ASH1 nor ASH2 were found to coimmunoprecipitate with BRM (Fig. 5C). We therefore conclude that the ASH1 and ASH2 proteins do not stably interact with the BRM complex.

To determine whether ASH1 and ASH2 are components of protein complexes distinct from the BRM complex in the *Drosophila* embryo, we examined the native molecular mass of both proteins by gel filtration chromatography. Western blotting revealed that the ASH1 protein elutes from a Superose 6 FPLC column with a native molecular mass of approximately 2 MDa (Fig. 6, fractions 17-23). By contrast, ASH2 was found to elute in fractions 27-29 with an apparent native molecular mass of approximately 500 kDa (Fig. 6). No monomeric ASH1 or ASH2 was detected in embryo extracts. Taken together, these data allow us to conclude that the *Drosophila* embryo contains at least three distinct protein complexes containing trx-G proteins: the 2 MDa BRM complex, a 2 MDa ASH1 complex and a 500 kDa ASH2 complex.

Genetic identification of proteins that interact with *brm* in vivo

As an alternative approach to identifying genes that are critical for *brm* function in vivo, we screened for mutations and deficiencies that are lethal in combination with an engineered, dominant-negative *brm* transgene, *brm*^{K804R}. The *brm*^{K804R} mutation is a point mutation that converts a conserved lysine in the ATP-binding site to an arginine. This mutation eliminates the activity of the BRM protein without affecting its interactions with other proteins. When expressed under the control of the *brm* promoter, the *brm*^{K804R} transgene has a strong, dominant-negative effect on *brm* function in vivo (Elfring et al., 1998). Individuals bearing one or two copies of an autosomal *brm*^{K804R} transgene exhibit a mild haltere-to-wing transformation due to decreased transcription of the homeotic gene *Ubx*. Expression of higher levels of the BRM^{K804R} protein is lethal.

An unusual interaction between an X-linked *brm*^{K804R} transgene (*P[w*$^{+}$*, brm*^{K804R}*]22D*) and amorphic *brm* alleles provided a convenient genetic assay for identifying factors that

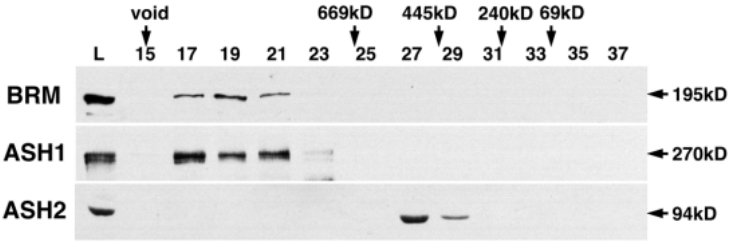

Fig. 6. ASH1 and ASH2 are members of distinct high molecular mass complexes. Native molecular masses of ASH1 and ASH2 were determined by Superose 6 gel filtration chromatography of embryo extracts. The denatured molecular masses of BRM, ASH1 and ASH2 based on molecular mass markers are indicated by arrows at right. Lanes are designated by the Superose 6 column fraction number or L for material loaded. Elution volumes of native molecular mass standards are indicated by arrows. Western blotting with the antibodies indicated at left revealed that the ASH1 and ASH2 proteins have native molecular masses of 2 MDa and 500 kDa, respectively.

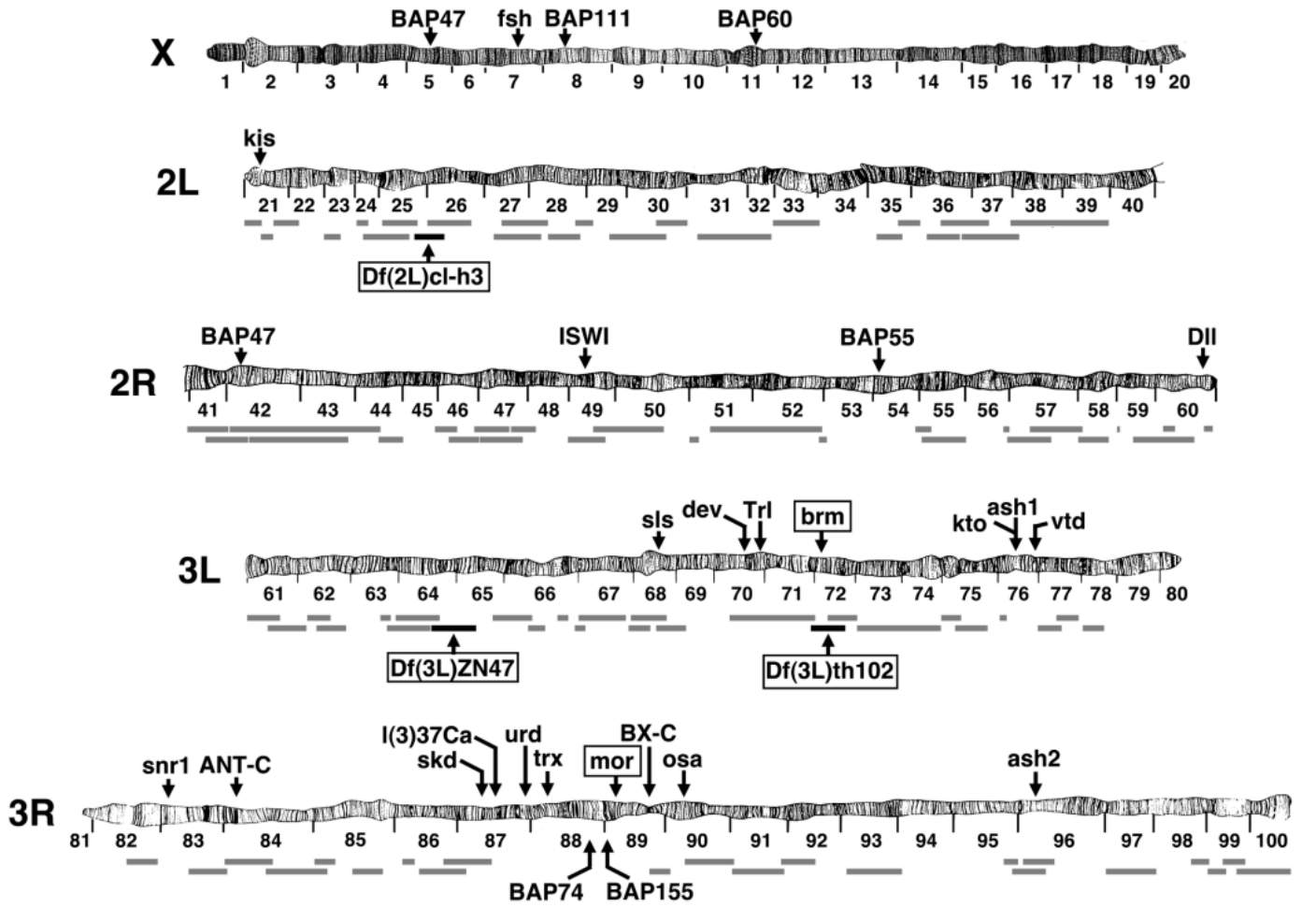

Fig. 7. Cytological positions of BAPs, trx-G genes and deficiencies tested for interactions with brm^{K804}. The regions missing in the deficiencies tested for interactions with the brm^{K804R} transgene are indicated by horizontal bars below the chromosomes. The cytological positions of BAPs and trx-G genes are indicated by arrows. Boxed text identifies deficiencies (also noted by black bars) and trx-G genes that interact with the brm^{K804R} transgene.

interact with *brm*. Female *brm* deficiency heterozygotes bearing a single copy of $P[w^+, brm^{K804R}]22D$ survive to adulthood, but males of this genotype are not viable (Table 2). This is due to dosage compensation of X-linked genes in males, which causes a two-fold increase in the level of the dominant-negative BRM protein. In this sensitized background, a two-fold reduction in the level of wild-type BRM protein is lethal. We therefore reasoned that mutations in other genes that are essential for *brm* function might also cause male-specific lethality in combination with the X-linked brm^{K804R} transgene.

To determine whether this approach for identifying genes that interact with *brm* is sensitive but selective, we screened a collection of deficiencies that together span approximately 60% of the autosomal genome for interactions with the brm^{K804R} transgene (Fig. 7). Only four of the 118 deficiencies tested (*Df(3L)th102, Df(3L)brm11, Df(3L)ZN47 and Df(2L)cl-h3*) caused male-specific lethality in combination with $P[w^+, brm^{K804R}]22D$ (Table 2; Fig. 7). *Df(3L)th102* and *Df(3L)brm11* both span the *brm* gene in polytene chromosome region 72A. The other two deficiencies, *Df(3L)ZN47 and Df(2L)cl-h3*, delete polytene chromosome regions 64C;65C and 25D2,3;26B2-5, respectively. *Df(3L)v65c*, a deficiency that extends from 64E1-13 to 65C1-D6 and partially overlaps *Df(3L)ZN47*, also interacts with brm^{K804R}, which places the gene responsible for this interaction in the 64E1;65C1 region. Interestingly, none of the known trx-G genes or BAP-encoding genes identified in this study map to 25D2,3;26B2-5 or 64E1;65C1 (Fig. 7). This suggests that these regions contain novel genes essential for *brm* function in vivo. Having confirmed the selectivity of this assay, we next examined whether mutations in trx-G genes (including *dev, kis, mor, osa, skd, sls, ash1, ash2, trx, Trl, urd, snr1* and *vtd*) interact with the brm^{K804R} transgene (Table 2). Surprisingly, only mutations in *brm* and *mor* were found to cause male-specific lethality in combination with $P[w^+, brm^{K804R}]22D$, which suggests that the function of these two trx-G proteins is intimately related.

DISCUSSION

At least three different trx-G complexes are present in the *Drosophila* embryo

Based largely on genetic data, it has been proposed that trx-G proteins, including BRM, physically interact to activate the

Table 2. Interactions of selected deficiencies and trx-G mutations with the *brm^{K804}* transgene

	brm^{K804R} males		*brm^{K804R}* females		
	mutant	control	mutant	control	% survival
Df(3L)brm11	0	50	62	120	0.0
Df(3L)th102	0	279	359	439	0.0
Df(3L)ZN47	0	57	23	96	0.0
Df(3L)v65C	0	21	45	35	0.0
mor^{6}	0	49	112	131	0.0
mor^{5}	1	35	68	70	2.8
Df(2L)cl-h3	2	61	80	145	3.2
brm^{2}	5	79	99	84	6.0
vtd^{5}	41	76	106	120	35.0
sls^{1}	23	36	82	107	39.0
urd^{2}	45	65	134	194	40.9
kto^{1}	110	134	285	258	45.1
dev^{1}	65	71	144	81	47.8
osa^{1}	80	83	218	110	49.1
snr1^{r3}	55	52	70	65	51.4
skd^{2}	97	79	203	58	55.1
Pc^{3}	31	21	42	37	59.6
trx^{E2}	56	32	66	51	63.6
ash2^{1}	84	41	156	84	67.2
Trl^{R85}	59	26	52	63	69.4
kis^{1}	99	40	194	141	71.2
ash1^{22}	153	60	301	114	71.8

P[w^{+}, brm^{K804R}]22D virgin females were mated to males bearing a deficiency or trx-G mutation in *trans* to a balancer chromosome. For each mutation, the number of male and female *P[w^{+}, brm^{K804R}]22D* progeny bearing the trx-G mutation or deficiency (mutant) versus balancer (control) chromosome is shown. % survival refers to the number of male *P[w^{+}, brm^{K804R}]22D* progeny bearing the mutant chromosome relative to the total number of male *P[w^{+}, brm^{K804R}]22D* progeny. If this value was ~10% or lower, a mutant chromosome was considered to cause male-specific lethality in combination with *P[w^{+}, brm^{K804R}]22D*. Mutations shown are a subset of those tested and include deficiencies showing a strong interaction with the dominant negative *brm* transgene as well as representative alleles of all trx-G genes tested.

transcription of homeotic genes. Although the discovery that BRM is the ATPase subunit of a 2 MDa complex seemed to support this possibility, our results have clearly shown that the BRM complex is not a complex of trx-G proteins. None of the proteins associated with BRM correspond to known trx-G proteins and, with the possible exception of *BAP155*, none of the genes encoding BAPs map in the vicinity of known trx-G genes.

The biochemical analysis of two other trx-G proteins, ASH1 and ASH2, revealed the existence of two additional high molecular mass complexes containing trx-G proteins. *ash1* and *ash2* were identified in a screen for mutations that affect imaginal disc development and were subsequently shown to cause homeotic transformations due to the decreased transcription of homeotic genes (LaJeunesse and Shearn, 1995; Shearn et al., 1987). Mutations in *ash1* enhance mutations in *brm* and suppress mutations in *Polycomb*, suggesting that *brm* and *ash1* are functionally related (Shearn, 1989; Tripoulas et al., 1994). A possible interaction between *brm* and *ash2* was suggested by the discovery that mutations in these genes cause similar defects in the development of the adult sensory organs, including campaniform sensilla and mechanosensory bristles (Adamson and Shearn, 1996; Elfring et al., 1998). Although ASH1, ASH2 and BRM are subunits of distinct complexes, it remains possible that these complexes act in concert to activate transcription. Further analysis of the ASH1 and ASH2 complexes will be necessary to determine their mechanism of action and relationship to other trx-G proteins, including BRM.

Another candidate for a trx-G protein that interacts with BRM is TRX. Rozenblatt-Rosen et al. (1998) recently identified SNR1 in a yeast two-hybrid screen for proteins that interact with TRX. Recombinant TRX and SNR1 bind to each other in vitro and the two proteins can be coimmunoprecipitated from extracts of flies expressing high levels of SNR1 under the control of a heat-shock-inducible promoter. Although these data suggest that TRX may target the BRM complex to specific regulatory regions via a direct interaction with the SNR1 protein, it should be noted that the TRX protein has not been shown to physically associate with the BRM complex. We did not observe any polypeptides with molecular mass similar to that of TRX (>350 kDa) in the BRM complex purified from *Drosophila* embryos, indicating that TRX is not a major subunit of the BRM complex. We have also been unable to reproducibly coimmunoprecipitate the TRX and BRM proteins from *Drosophila* embryos expressing epitope-tagged BRM protein. These negative results may be due to the low abundance and instability of the large TRX protein. Alternatively, TRX may not interact with SNR1 in the context of the BRM complex.

The functions of the trx-G genes *brm* and *mor* appear to be intimately related

As an alternative approach for identifying trx-G proteins that are essential for BRM function in vivo, we screened for mutations that interact with a dominant-negative *brm* transgene, *brm^{K804R}*. Surprisingly, mutations in the trx-G genes *dev*, *kis*, *osa*, *skd*, *sls*, *ash1*, *ash2*, *trx*, *Trl*, *urd*, *snr1* and *vtd* failed to interact with *brm* in this assay. This suggests that there are important functional differences between *brm* and these trx-G genes. In contrast, we observed a strong genetic interaction between *brm* and and the trx-G gene *mor*. Both *brm* and *mor* were identified in a screen for extragenic suppressors of *Pc* (Kennison and Tamkun, 1988). Mutations in both genes cause homeotic transformations, including the transformation of haltere to wing due to decreased transcription of *Ubx* (Brizuela and Kennison, 1997; Elfring et al., 1998). Both genes are also required for oogenesis and the proper expression of the segmentation gene *engrailed* (Brizuela et al., 1994; Brizuela and Kennison, 1997). In the light of these genetic similarities, it is intriguing that *mor* is also the only trx-G gene that lies in the vicinity of a BRM complex subunit, BAP155.

The identification of a protein related to SWI3 and RSC8 (BAP155) in the BRM complex is consistent with the conservation of domain II in BRM. Two-hybrid studies demonstrated that domain II is part of a region that mediates an interaction of SWI2/SNF2 with SWI3 and of STH1 with RSC8 (Treich et al., 1995; Treich and Carlson, 1997). Deletion of domain II from BRM eliminates BRM function in vivo and decreases the native molecular mass of the BRM complex (Elfring et al., 1998). Based on these data, it is likely that BAP155 interacts with BRM via domain II.

Our analysis of deficiencies spanning approximately 60% of the autosomal genome identified a small number of deficiencies that cause male-specific lethality in combination with *brm^{K804R}*. These deficiencies are thus likely to contain genes that are essential for *brm* function in vivo. What is the

molecular basis of their genetic interaction with *brm*? Our genetic assay is based on the ratio of BRM to BRM^{K804R} protein. Since both proteins are expressed from identical promoters, the interacting genes in the 25D2,3;26B2-5 and 64E1;65C1 regions are likely to affect the function of the BRM protein, as opposed to its expression. No previously identified trx-G genes or genes encoding BAPs map to either of these regions. The genes within these regions that interact with *brm* could encode either substoichiometric subunits of the BRM complex or other proteins that indirectly or transiently interact with BRM in vivo. We are currently attempting to identify the genes within these deficiencies that interact with *brm*.

The BRM complex contains subunits common to the yeast SWI/SNF and RSC complexes

Based on genetic and biochemical data, we had proposed that BRM is the ATPase subunit of a conserved *Drosophila* counterpart of the yeast SWI/SNF complex. The characterization of proteins associated with BRM has revealed both similarities and differences between the BRM and SWI/SNF complexes. Four of the eight major subunits of the BRM complex – BRM, BAP155, BAP60 and BAP45/SNR1 – are equally related to subunits of both the SWI/SNF and RSC complexes. The presence of these four subunits in the BRM complex suggests that it counteracts the repressive effects of nucleosomal histones on transcription. However, the BRM complex lacks subunits related to SWI/SNF-specific subunits (including SWI1, SNF6 or SNF11) and no ESTs corresponding to potential *Drosophila* homologs of these proteins have been identified. Other differences between BRM and SWI/SNF have also been reported. For example, SWI/SNF is neither abundant nor essential, while BRM is essential for cell viability and is expressed at relatively high levels (approximately one molecule per twenty nucleosomes) (Elfring et al., 1998). These findings suggest that there are likely to be functional differences between the *Drosophila* BRM and yeast SWI/SNF complexes. Is the BRM complex a *Drosophila* counterpart of RSC? Like RSC, the BRM complex is both essential and relatively abundant. However, the BRM complex has far fewer subunits than RSC (eight versus fifteen) and appears to lack polypeptides related to RSC-specific subunits (B. Cairns, personal communication). It therefore appears that *Drosophila* contains a single chromatin remodeling complex that is equally related to SWI/SNF and RSC, but a clear homolog of neither complex.

The BRM complex is highly related to the human BRG1 and hBRM complexes

As anticipated, the BRM complex bears greatest similarity to the human BRG1 and hBRM complexes. Biochemical studies of the BRG1 and hBRM complexes have identified multiple proteins related to subunits of the SWI/SNF and RSC complexes: two are related to the SWI2/SNF2 and STH1 proteins (BRG1 and HBRM); three are related to the SWP73 and RSC6 proteins (BAF60a, BAF60b and BAF60c); two are related to the SWI3 and RSC8 proteins (BAF155 and BAF170); and one is related to the SNF5 and SFH1 proteins (BAF47/INI1) (Table 1). What is the significance of this subunit heterogeneity? In some cases, related subunits may be used in a tissue-specific fashion. For example, human BAF60c is expressed at highest levels in muscle cells while human BAF60a and BAF60b are expressed in many tissues (Wang et al., 1996b). In other cases, related subunits are simultaneously present in a single complex e.g. the human proteins related to SWI3 (BAF155 and BAF170) coexist in a single BRG1 or hBRM complex (Wang et al., 1996b).

The BRM complex contains proteins that are highly related to each of the previously identified subunits of the BRG1 and hBRM complex, including the subunits conserved in SWI/SNF and RSC and the HMG-domain protein that is unique to higher eukaryotes. This striking degree of conservation suggests that the BRM complex is functionally homologous to the human BRG1 and hBRM complexes. In contrast to the situation in humans, however, we found that each subunit of the BRM complex is encoded by a single copy gene. While we cannot rule out the existence of divergent relatives of the BAPs, no highly related gene families exist in *Drosophila*. *Drosophila* thus contains a single SWI/SNF-like complex that lacks the subunit heterogeneity of its human counterparts.

In vitro assays for chromatin remodeling have failed to detect any differences between the SWI/SNF, RSC, hBRM and BRG1 complexes despite the differences in their subunit composition. This implies that the four conserved subunits are responsible for their in vitro activities while the majority of the subunits confer specificity or as yet unidentified functions relevant in vivo. One such subunit is the HMG domain protein which appears to be unique to the higher eukaryotic complexes (BAF57 and BAP111). Binding of HMG domain proteins can bend, loop and cross DNA, recognize or stabilize such structured DNA, and affect supercoiling of constrained DNA. By inducing or recognizing structured DNA, HMG domain proteins are believed to facilitate the binding or interaction of other DNA-binding proteins in vivo (Zlatanova and van Holde, 1998). Do the HMG domain proteins in the BRM, hBRM and BRG1 complexes mediate interactions between these complexes and DNA? Although recombinant BAF57 protein is able to bind 4-way junction DNA, human complexes containing a mutant BAF57 protein lacking the HMG domain are still able to bind 4-way junction DNA in vitro (Wang et al., 1998). This result suggests there must be another subunit performing a similar function in the human complexes. Genetic studies of *Drosophila* BAP111 should determine whether the HMG domain of this protein is essential for the function of the BRM complex.

Does the BRM complex directly counteract Polycomb repression?

The subunit composition of the BRM complex suggests that it is likely to stimulate the binding of transcription factors to nucleosomal DNA in vitro, as has been demonstrated for the SWI/SNF, RSC, BRG1 and hBRM complexes. Given the tremendous size and subunit complexity of these molecular machines, it seems unlikely that they function solely to counteract the repressive effects of nucleosomes. The recovery of *brm* mutations in a screen for extragenic suppressors of *Pc* mutations has suggested that one function of the BRM complex may be to counteract the repressive effects of Pc-G proteins on ANT-C and BX-C genes. However, no physical interaction between a subunit of the BRM complex and a Pc-G protein has yet been observed and it is possible that BRM does not directly counteract Pc-G repression. The further analysis of *brm* and other trx-G and Pc-G proteins will be required to address this

issue and clarify how these conserved eukaryotic regulatory proteins interact to maintain stable states of transcriptional activation and repression.

We thank Kathy Matthews and the Bloomington *Drosophila* Stock Center for providing numerous stocks, Andrew Dingwall (Syracuse University), Arno Greenleaf (Duke University), Peter Harte (Case Western Reserve University), Alexander Mazo (Thomas Jefferson Medical College) and Carl Wu (NCI) for providing antibodies, and William S. Lane, Renee Robinson, John Neveu, Dan Kirby, Kerry Pierce, and Eric Spooner of the Harvard Microchemistry Facility for peptide sequencing. The authors would also like to thank Bradley Cairns, Gerald Crabtree, Gary Daubresse, Matthew Scott and Weidong Wang for sharing unpublished information. We are grateful to Andrew Chisholm, Yishi Jin, Matthew Scott, Jeff Simon, John Sisson and William Sullivan for comments on the manuscript. This work was supported by a grant from the National Institutes of Health (GM49883) to J. W. T.

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