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Transcriptional activation in *Drosophila* spermatogenesis involves the mutually dependent function of *aly* and a novel meiotic arrest gene *cookie monster*

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SUMMARY

In *Drosophila* spermatogenesis, meiotic cell cycle progression and cellular differentiation are linked by the function of the meiotic arrest genes. The meiotic arrest genes control differentiation by regulating the transcriptional activation of many differentiation-specific genes. The meiotic arrest genes have been subdivided into *aly* and *can* classes, based on the mechanism by which they control cell cycle progression. *aly* has previously been shown to encode a chromatin-associated protein. We present the identification, cloning and characterisation of a novel *Drosophila* meiotic arrest gene, *cookie monster* (*comr*), that has a mutant phenotype indistinguishable from that of *aly*. A null mutant allele of *comr* is viable but male sterile. Mutant primary spermatocytes fail to initiate transcription of a large number of genes, and arrest before entry into the meiotic divisions. In adult males, expression of *comr* is testis specific, low levels of transcripts are detected at other stages of development. *comr* encodes a novel acidic protein, which is nuclear and primarily localised to regions of chromatin in primary spermatocytes. The nuclear localisation of Aly and Comr proteins are mutually dependent. Finally, we show that active RNA polymerase II is found in distinct domains in the nucleus that constitute a subset of the total Comr stained chromatin.

Key words: *Drosophila*, Meiosis, Transcription, Spermatogenesis, Nuclear localisation

INTRODUCTION

Differential control of gene expression during development is crucial for the specification and maintenance of differentiated cell types. One of the most dramatic switches in the pattern of gene expression occurs during spermatogenesis, many genes are switched on only in this cell type. Some are germline-specific homologues of ubiquitously expressed genes (e.g. *β2 tubulin*) (Kemphues et al., 1982) and the mitochondrial fusion protein *fzo* (Hales and Fuller, 1997), others are spermatogenesis specific proteins, e.g. the protamines that replace histones to tightly package sperm DNA (Peschon et al., 1987). Even genes transcribed in other tissues often have a separate, testis-specific, enhancer element in their promoter region, e.g. *gonadal* (Schulz et al., 1990).

In *Drosophila* spermatogenesis, transcription is essentially shut off upon entry into the meiotic divisions, therefore all transcripts required at later stages must be made in primary spermatocytes (Olivieri and Olivieri, 1965). Four mitotic divisions of a spermatogonial cell produce a cyst of 16 primary spermatocytes, which immediately undergo premeiotic S phase. They then enter an extended G2 period, characterised by high transcriptional activity and cell growth. The meiotic divisions result in a cyst of 64 round spermatids, which use stored mRNAs to produce proteins needed for their dramatic morphological changes during elongation, before finally individualising to form motile sperm (reviewed by Fuller, 1993).

Although entry into spermatid differentiation is independent of progression through the meiotic divisions, these processes are subject to coordinate control, mediated by the 'meiotic arrest' class of genes, including *aly*, *can*, *mia* and *sa* (Lin et al., 1996), and several other unpublished mutations (M. Fuller, personal communication). The meiotic arrest genes are essential for the transcription of many mRNAs involved in spermatid differentiation, and thus are required for spermatid differentiation. The meiotic arrest genes also control accumulation of proteins involved in the meiotic divisions, e.g. the *cdc25* homologue Twine, and thus link differentiation to the cell cycle (White-Cooper et al., 1998). The meiotic arrest genes of *Drosophila* have been split into two classes, based on the mechanism by which they control accumulation of Twine. The *can* class (including *can*, *mia* and *sa*) post-transcriptionally regulate Twine production. By contrast *aly* regulates transcription of *twine*. Two other meiotic regulators, *cyclin B* and *boule*, are also transcriptional targets of *aly*, but not *can*, *mia* or *sa*.

Three meiotic arrest genes have been cloned to date, *aly*, *can* and *nht* (Hiller et al., 2001; White-Cooper et al., 2000). *can* and *nht* encode testis-specific homologues of $TAF_{II}80$ and

$TAF_{II}110$, respectively. These are subunits of the basal transcription factor $TF_{II}D$, whose role is to facilitate RNA polymerase II binding to the proximal promoter region. The *aly* (*always early*) gene discussed in this paper should not be confused with the regulator of RNA export *Aly* (Zhou et al., 2000). *aly* encodes a homologue of a *C. elegans* negative regulator of vulval induction, the SynMuvB gene *lin-9*. The SynMuv B pathway includes several genes whose products form a complex (NURD) that regulates chromatin structure (Lu and Horvitz, 1998; Solari and Ahringer, 2000).

The mechanism by which *lin-9* regulates the NURD complex is not understood. LIN-9 has not been shown to be a component of the NURD complex itself, so may therefore act upstream. The subcellular localisation of LIN-9 in *C. elegans* has not been determined. We previously showed that Aly protein localised to chromatin in maturing *Drosophila* primary spermatocytes. This localisation suggests that the *lin-9* homologue, Aly, may act in close concert with a NURD complex on chromatin (White-Cooper et al., 2000). The nuclear localisation of Aly protein is both regulated and essential for the normal function of the protein, as the protein produced by several mutant alleles remains cytoplasmic, despite the presence of a nuclear localisation signal.

We report the identification and cloning of *cookie monster* (*comr*), a novel *aly*-class *Drosophila* meiotic arrest gene. Like *aly*, *comr* transcription is testis specific in males, but low levels of transcript were detected at earlier stages of development. We show that Comr protein is localised to the nucleus of primary spermatocytes, and concentrated on decondensed regions of chromatin. The Comr pattern is similar but not identical to that of Aly. We show that the nuclear localisation of these two proteins is mutually dependent. Finally, we show that active RNA polymerase II is limited to discrete regions of the nuclei of primary spermatocytes. These regions of high transcriptional activity are a subset of the Comr localisation domain, but the level of Comr protein does not predict the level of active RNA polymerase II.

MATERIALS AND METHODS

Drosophila culture and markers

Drosophila were maintained on standard cornmeal/agar/sucrose medium at 25°C. All the markers used here have been described previously (Lindsley and Zimm, 1992) and at FlyBase (FlyBase, 1999). Deficiency chromosome lines were obtained from Bloomington Stock Centre. Wild-type flies were Oregon R for molecular biology or *red e* for immunostaining.

Isolation of a *comr* mutant allele

A large scale mutagenesis screen to isolate new viable mutants induced by EMS on second chromosomes marked with *cn* and *bw*, was conducted by Charles Zuker and colleagues (E. Koundakjian, R. Hardy, D. Cowen and C. Zuker, personal communication). All the lines generated were tested for male sterility by Barbara Wakimoto and Dan Lindsley, who then conducted a preliminary morphological examination by phase-contrast microscopy to determine the defect in each male sterile line. These lines were re-screened in Margaret Fuller's laboratory, and those with a meiotic arrest phenotype were tested inter se for allelism. *comr* corresponds to line *Z2-1340*, and was represented by a single mutant allele. Mutant phenotypes were scored by squashing cut testes in testis buffer (183 mM KCl, 47 mM NaCl, 10 mM Tris pH 6.8) and observing with phase contrast optics. Hoechst 33342 (4 μg/ml) was included in the dissection buffer to visualise chromosomes in live spermatocytes.

PCR from flies

Df(2R)Egfr3/*Df(2R)X58-7* and *Df(2R)XE2900*/*Df(2R)X58-7* males were selected from crosses based on the lack of the balancer chromosome. Testes of some mutant males were dissected to confirm the mutant phenotypes, the remaining mutant flies were used for genomic DNA extraction. Genomic DNA was simultaneously extracted from wild-type male flies, and from flies homozygous for a different mutation generated in the same mutagenesis screen. These DNAs were used as PCR templates to amplify the following candidate genes predicted by the genome sequencing and annotation projects: CG9284, CG4386, CG13492, CG4363, CG4377, CG4372, CG9294, CG13493 and PpN58A (Adams et al., 2000).

Sequencing of candidate genes

PCR primers were designed to amplify all of the predicted ORF from candidate genes, CG4363, CG4377, CG4372, CG9294 and CG13493 as sets of overlapping products. The amplified fragments were sequenced from both ends using BigDye terminator cycle sequencing reagent (ABI), reactions were run on a ABI 377 automated DNA sequencing system. Sequence alignments were carried out using Sequencher 3.1 (GeneCodes Corp).

RT-PCR of *comr*

Total RNA was prepared using TRIzol reagent (Roche) from testes of wild-type male flies, the whole bodies of wild-type males and females respectively, and from wild-type male carcasses (testes were removed by dissection). The total RNA was treated by DNAse I to remove possible contamination of genomic DNA. RT-PCR was carried out using Superscript Preamplificaion System (Invitrogen). A pair of oligo primers (5′-GATTACCAGGGTATGCAGGA and 5′-GGCTTTGCTT-TAAACCTGGT) was used to amplify a 979 bp cDNA fragment from the open reading frame of *comr*. Lack of DNA contamination was confirmed by RT-PCR of the ubiquitously expressed gene PP1-87B using primers that spanned an intron.

In situ hybridisation

The 979 bp *comr* RT-PCR product was subcloned into pBluescript (Stratagene). pBluescript clones containing *cyclin B*, *cyclin A*, *Mst87F*, *boule* and *polo* were prepared as previously described (White-Cooper et al., 1998). Antisense dig-labelled RNA probes were made using the Roche Dig-RNA labelling mix, according to the manufacturer's instructions. Hydrolysis was for 15 minutes per 500 bp. Hybridisation was carried out as previously described (White-Cooper et al., 1998) .

Antibody production

Anti-peptide antibodies were raised by Moravian-Biotechnology. The synthesised oligo-peptide, KSRRYDLRNSKRNPR (amino acids 586-600) at the C terminus of the predicted Comr protein, was coupled to BSA and used to immunise a rabbit. The serum was preadsorbed against fixed *Drosophila* ovaries to deplete non-specific or low affinity antibodies. Rabbit anti-Comr was used at a dilution of 1:5000 for western blotting.

Western blotting

Testes were dissected from young males in testis buffer and transferred to an Eppendorf tube and snap frozen in liquid nitrogen. They were thawed, 5×SDS sample buffer added and the samples were boiled for 5 minutes then spun in a microfuge for 5 minutes. Typically 20 testes were loaded on each lane of a 10% SDS-PAGE gel. Proteins were transferred onto a nitrocellulose membrane, which was then blocked in 5% fat-free powdered milk in PBS+0.1% Tween-20. Primary and HRP-conjugated secondary antibodies were diluted in blocking solution and incubated with the blot overnight at 4°C and

room temperature for 1 hour, respectively. Signals were detected using Pierce SuperSignal chemi-luminescence reagent and Kodak MBX blue sensitive X-ray film.

Immunohistochemistry and Immunofluorescence

For immunohistochemistry, testes were dissected from 0 to 1-day-old males in testis buffer and fixed in 4% paraformaldehyde in HEPES buffer (0.1 M HEPES pH 6.9, 2 mM $MgSO_4$, 1 mM EGTA) for 30 minutes. The testes were rinsed once then transferred to cell culture inserts (Falcon) in a 24-well tissue culture plate, washed three times for 20 minutes each in PBS+0.1% Triton-X100 (PBSTx), blocked for 30 minutes in PBSTx+5%FCS, then incubated overnight at 4°C or 4 hours at room temperature in primary antibody diluted in this blocking buffer. After four 15 minute washes in PBSTx the testes were incubated 2 hours at room temperature or overnight at 4°C with Universal biotinylated secondary antibody (Vector), preadsorbed against *Drosophila* embryos and diluted 1:1000 in blocking buffer. After four 15 minute washes in PBSTx, the testes were incubated for 1 hour at room temperature with ExtrAvidin-HRP (Sigma), diluted 1:1000 in PBSTx. Testes were washed three times for 15 minutes each in PBSTx, rinsed once in PBS then stained with diamino-benzidine (0.5 mg/ml) + 0.001%H_2O_2 in PBS. The colour reaction was monitored periodically and stopped by washing with PBS. Testes were mounted in 85% glycerol and observed with an Olympus BX50 microscope equipped with DIC optics. Rabbit anti-Aly was diluted 1:4000 and rabbit anti-Comr was preadsorbed against ovaries and diluted 1:1000.

For immunofluorescence, testes were dissected from 0-1 day old males in testis buffer, about five males were used per slide. The testes were transferred to a 25 µl drop of testis buffer on a poly-l-lysine-treated slide and cut open with tungsten needles. Paraformaldehyde (25 µl of 4% solution) in HEPES buffer was added and the testes were left to fix for 12 minutes at room temperature. Testes were squashed by adding a coverslip, the slide was dunked in liquid nitrogen and the coverslip was removed with a scalpel. Testes were then stored in PBS+0.1% Tween-20 (PBSTw) until all the samples had been prepared. Samples were blocked for 30 minutes with PBSTw+ 5%FCS, then incubated with primary antibody diluted in blocking solution overnight at 4°C. Testes were rinsed once then washed four times for 10 minutes each in PBSTw. Testes were incubated with secondary antibodies diluted in blocking solution for 2 hours at room temperature. When DNA was to be stained with propidium iodide, RNAse A (0.5 mg/ml) was included with the secondary antibody incubation. Samples were washed as before and mounted in 85% glycerol + 2.5% n-propyl gallate. For DNA staining propidium iodide (1µg/ml) was included in the mounting medium. Coverslips were sealed with nail varnish and the samples were imaged using a BioRad Radiance Plus confocal microscope.

The monoclonal antibody against RNA polymerase II phospho-C-terminal domain (P-CTD) (Clone H5) was obtained from Covance/BAbCO and used at a dilution of 1:100. Pre-adsorbed anti-Comr antibody was used at 1:1000. Secondary antibodies coupled to Cy3 or FITC were used at 1:1000 (Jackson).

RESULTS

comr is a novel *aly*-class meiotic arrest gene

A large collection of EMS-induced male sterile mutations (see Wakimoto, 2000) were examined by phase-contrast microscopy of testes squashes to identify potential new meiotic arrest loci. Wild-type testes contain cysts of cells at many different stages of spermatogenesis: pre-meiotic, meiotic and post-meiotic (Fig. 1A). By contrast, testes from one of the Zuker lines (*Z2-1340*) contained mature pre-meiotic primary spermatocytes, but no cysts undergoing meiotic divisions or attempting post-meiotic spermatid differentiation (Fig. 1B). *Z2-1340* testes were smaller than wild type because of the lack of later stages of spermatogenesis. The arrested cells degenerated towards the distal end of the mutant testes. *Z2-1340* females were fertile. As *Z2-1340* maps to the second chromosome, and all of the previously characterised meiotic arrest genes were on the third chromosome, *Z2-1340* must represent a previously uncharacterised meiotic arrest locus, which we call *cookie monster* (*comr*) because the cells look like a 'whole bunch of cookie monster eyes'.

All of the four previously described meiotic arrest loci show defects in transcription in primary spermatocytes of many genes required for spermatid differentiation, including *Mst87F* and *fzo*. The meiotic arrest genes were subdivided into *aly* class and *can* class because certain cell cycle genes, namely *cyclin B, twine* and *boule* were transcribed in *can*-class mutants (*can, mia* and *sa*) but not in *aly* (White-Cooper et al., 1998). A set of diagnostic RNA in situ hybridisation experiments revealed that *comr* resembled *aly* rather than the *can*-class mutant *mia* (Fig. 2). *comr* mutant testes transcribed *polo*, but failed to transcribe both *boule* and *Mst87F*.

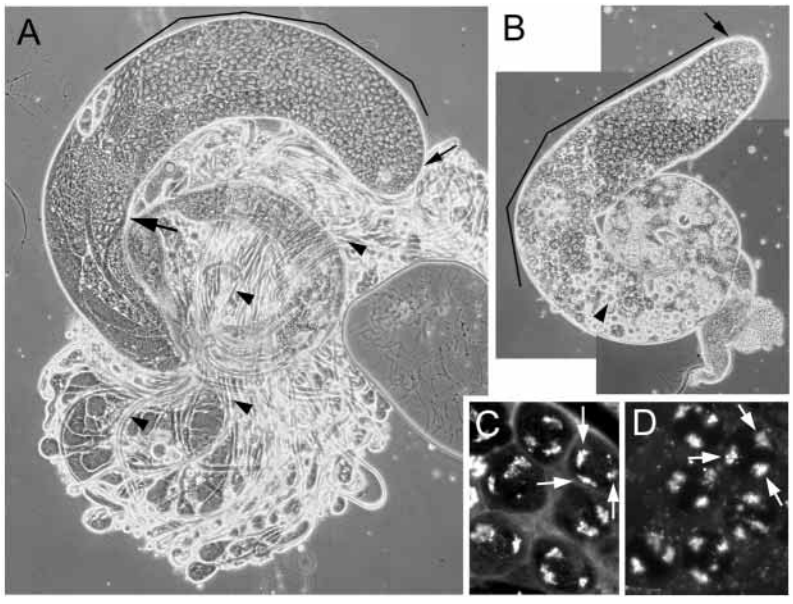

Fig. 1. $comr^{z1340}$ testes show a meiotic arrest phenotype. (A,B) Phase contrast micrographs of wild-type (A) and $comr^{z1340}$ (B) mutant testes. Wild-type testis contains stages of spermatogenesis from mitotic spermatogonia (small arrow), primary spermatocytes (in region indicated by black line adjacent to testis), meiosis (large arrow) and spermatid elongation (arrowheads). $comr^{z1340}$ testes contain mitotic spermatogonia (small arrow) and primary spermatocytes (line). Later stages are absent; instead, the basal region of the testis contains refractile necrotic cells (arrowhead). (C,D) Chromosomes of live wild-type (C) and $comr^{Z1340}$ (D) primary spermatocytes visualised by staining with vital Hoechst. The arrows indicate the three major chromosome bivalents within one nucleus. The mutant chromosomes look fuzzy compared with wild type.

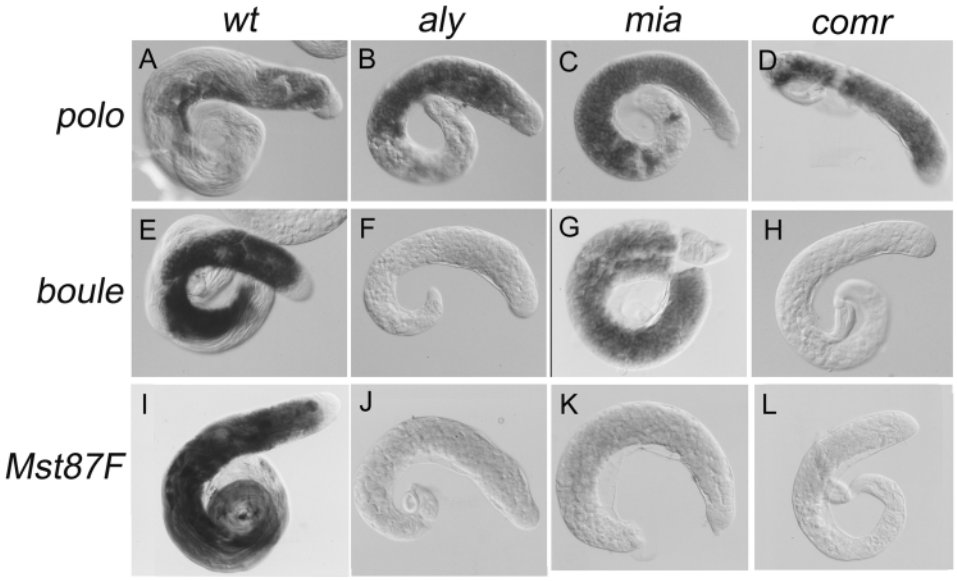

Fig. 2. *comr* is more like *aly* than the *can*-class meiotic arrest genes. RNA in situ hybridisation of wild-type (A,E,I), aly^{5} (B,F,J), *mia* (C,G,K) or $comr^{z1340}$ (D,H,L) testes, using probes against *polo*, *boule* and *Mst87F*, as indicated. Whole testes are shown, with the apical regions to the right-hand side of each panel; dark grey staining indicates presence of the transcript. Wild-type testes expressed *polo* in primary spermatocytes (A); *polo* mRNA was present in all the mutant testes (B-D). *boule* mRNA was expressed in wild-type primary spermatocytes, and the transcript persisted into early elongation stages (E). *boule* transcript was visible in *mia* primary spermatocytes (G), but was not detected in aly^{5} or $comr^{z1340}$ mutant testes (F,H). *Mst87F* mRNA accumulated in wild-type primary spermatocytes and persisted until late stages of elongation (I). No accumulation of *Mst87F* transcript could be detected in any of the mutant testes (J-L).

The phenotype of *comr* also resembled *aly* rather than the *can*-class genes in terms of the chromosome morphology in mutant primary spermatocytes, after staining with vital Hoechst (33342). *can*-class mutants had apparently normal chromosome morphology whereas *aly* had defects in chromatin structure. The major chromosome bivalents of wild-type primary spermatocytes formed three discrete, clearly delineated domains within each nucleus (Fig. 1C). By contrast the chromosome bivalents of $comr^{Z1340}$ mutant primary spermatocytes were fuzzy, with indistinct boundaries (Fig. 1D). *comr* mutant spermatocytes therefore resemble *aly* rather than *can* based on their failure to express mRNA for certain cell cycle control genes and their aberrant chromosome morphology. Thus, *comr* is the second member of the *aly*-class meiotic arrest genes of *Drosophila*.

Cloning of *comr*

Meiotic recombination mapping localised *comr* genetically to 96.0±5.7, between *c* and *px* on the right arm of chromosome 2. Complementation tests with deficiency chromosomes placed the *comr* locus in the physical region 57F-58A (Fig. 3). The left-hand end of the region is defined by the proximal break point of deficiency *Df(2R)X58-7*, which is reported as 58A1-2 (Kerrebrock et al., 1995). The right-hand end is defined by the distal breakpoint of *Df(2R)Egfr3*, reported as 57F5-11 (Price et al., 1989). Both these deletions uncovered *comr*, suggesting that they overlap. Although *Df(2R)Egfr3/Df(2R)X58-7* flies were viable and female fertile they were male sterile, indicating that they do indeed overlap. The testes of transheterozygous males had a phenotype indistinguishable in squash preparations from $comr^{Z1340}/comr^{Z1340}$ or $comr^{Z1340}/Df$. The boundaries of the overlapping deleted region were identified by PCR of predicted genes from transheterozygous *Df(2R)Egfr3/Df(2R)X58-7* males. PCR products were generated for CG4386, CG9284 and PpN58A from this template DNA, indicating that these genomic regions were not disrupted in at least one of the two deletion lines. By contrast, no PCR products were generated for the predicted genes

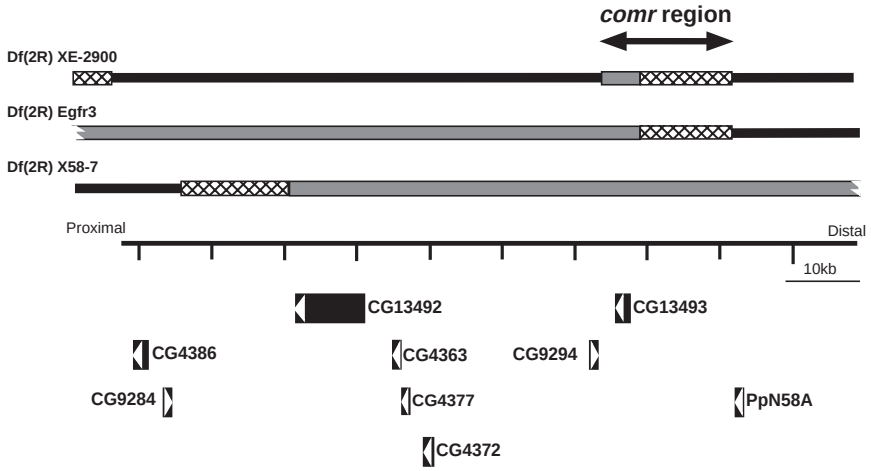

Fig. 3. *comr* genomic region showing overlapping deficiencies and predicted genes. The genomic region 57F-58A is shown in the centre of the figure. Deficiency chromosomes are shown above the region. Grey indicates deleted segments; black indicates non-deleted chromosomal regions; hatched boxes represent uncertainty. The predicted genes from this region are shown underneath: black boxes indicate the extent of the predicted gene; arrows indicate orientation. *comr* mapped to the overlap of *Df(2R)EGFR3* and *Df(2R)X58-7*, between CG9284 and PpN58A. A small deletion was detected in *Df(2R)XE-2900*, which uncovers CG13493. *Df(2R)XE-2900* also deletes a larger genomic region just proximal to that shown in this figure (indicated by the hatched box).

A

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MSGNQDTLGQ SQQVNESGDE YEEDAATSWT NELEQQESRT PADSPEFLDF   50
VDSEDPLEDF ERNPLQKEPL DLAVKPVSDI NKKAAENHRR IYRQLGIHIP  100
ETPKSHPLLH YAFVVKLFLA TDNSSIHYLK WSANGRELEV DYQGMQEHLS  150
SGCSMFHCRN TLQFTGTLLA HGFERVSLKD VRKSSATNIT LIYRNPNFVK  200
GQLLKLHKLA QECEKIPEIS SPGTAEPRRP PLHLPNYAHI SRMVRRGDLC  250
STPYTSRSAL QVARCHFQTL LGYQADLGVL KDRNNHDMFN KNIMTQLNVK  300
RGRSSINNAP VDLAPSKVSF EKFVKPHESV INIGIGQAPD YAGYYGQVEL  350
FKVNEFFSEY LPRYGSKITG YKEIVMDATN KSIGFQQNLP IGMNYSDDED  400
DALPPISSDL DLDFMPSTSA AAKMKSTGRK VVEDSDLEQA MQELCGGSNL  450
NEEEELPQTS TRFKAKPKPR AKTKAKPTKR QAKPVHSSSS ECEDFKPDEV  500
YEGFKSRNPK NVKLKPSDEN ISYTLEEGTD FEEQVIEETT ETKVQDSQYD  550
DLDDDDDDEE EDYFDEDDED YVYKNLQYRN PPEPPKSRRY DLRNSKRNPR  600
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B

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KPCI   QVRVKAYYRIMITHFES-IS-FEGLCNEVR-DMCSFDNEQLFTMKWIDEEFDPCT-VS
        .:|: ::   :.:: | |: :: :  :.  .  .|:::  : |:: |:| |. . :|
COMR   LFKVNEFFSEYLPRYGSKITGYKEIVMDATNKSIGFQQNLPIGMNYSDDEDDALPPIS

KPCI   SQLELEEAFRLYELNKDSELLIHVF
       |:|:|:         | ..   :|
COMR   SDLDLDFMPSTSAAAKMKSTGRKVV
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Fig. 4. Comr predicted protein sequence. (A) Conceptual translation of Comr protein. The mutated amino acid in *comr*^{z1340} Q12* is indicated in an oval. The very acidic region at the C terminus of the protein is double underlined. Putative nuclear localisation signal is single underlined. The potential PB1-like domain is indicated in italics. Peptide used for antibody production is indicated in bold. (B) ClustalW alignment of the PB1 domain from human KPCI with the PB1-like region from Comr. Identical residues are indicated with vertical lines, very conservative substitutions with double dots, conservative substitutions with single dots.

CG13492, CG4363, CG4377, CG4372, CG9294 and CG13493, indicating that these loci are disrupted by both deletions. Clearly none of these six loci is required for viability or female fertility, but at least one is required for male fertility. A third deletion, *Df(2R)XE-2900*, which deletes from 57F2-58A1 also uncovered *comr*^{Z1340}. Additionally *Df(2R)X58-7/Df (2R)XE-2900* flies were viable, but the males had a phenotype indistinguishable in squash preparations from *comr*^{Z1340}/*comr*^{Z1340}. PCR from these transheterozygotes revealed that CG13493 was deleted in *Df (2R)XE-2900*, but none of the other candidate genes was deleted, making CG13493 the strongest candidate for the *comr* gene. The open reading frame of CG13493 was sequenced from wild-type, *comr*^{Z1340} and another mutant derived from the Zuker screen to represent the background chromosome. *comr*^{Z1340} contained a single base change (C-T) in CG13493, which was not present in the background chromosome. This mutated codon 12 (CAG) to a stop codon (TAG), presumably resulting in a null allele. No coding region mutations were found in any of the other candidate genes sequenced. Thus CG13493 is *comr*.

The *comr* ORF, based on genome project predictions and RT-PCR (see below) encodes a novel 600 amino acid protein, with a predicted molecular weight of 68.4 kDa, and a predicted pI of 5.1 (Fig. 4A). The Comr protein showed no significant homology to any protein in the protein or translated EST databases. The predicted protein contained an acidic domain in the C terminus of the protein (amino acids 518-570), and a predicted nuclear localisation sequence (NLS) (amino acids 583-589). In addition, a region that may represent a very divergent PB1 domain (amino acids 348-431) was identified (Fig. 4B). PB1 domains have been found in several signal transduction proteins, including kinase C iota (KPCI), and have been shown to mediate protein-protein interactions (Ito et al., 2001; Ponting et al., 2002).

Expression of *comr*

comr was represented only once in the *Drosophila* EST database (of 246620 EST sequences), this cDNA (AT27686) is derived from a testis library and contains a 65 bp 5′UTR. The developmental expression profile of *comr* was determined by RT-PCR. The transcript was testis specific in adult males, being undetectable in gonadectomised males (Fig. 5A), consistent with the fully viable but male sterile phenotype of the mutant. Low levels of the transcript were also detected in whole females (Fig. 5A), embryos and larvae (data not shown). RNA in situ hybridisation revealed a uniform transcript distribution in embryos (data not shown).

In testes, the *comr* transcript was expressed at highest levels in early primary spermatocytes, as revealed by RNA in situ hybridisation. The transcript level decreased as the spermatocytes grew, and became undetectable as the primary spermatocytes entered the meiotic divisions (Fig. 5B). This expression pattern of *comr* is essentially identical to the pattern we have previously reported for *aly* (White-Cooper et al., 2000). The earliest defect we have detected in *comr* or *aly* mutant testes is failure to initiate transcription of target genes in very early primary spermatocytes. The strong expression of *comr* transcript in early primary spermatocytes is consistent with this phenotype.

Subcellular localisation of Comr protein

An antibody raised against a peptide from the C terminus of Comr protein recognised a 100 kDa protein in wild-type testes that was absent from *comr*^{Z1340} mutant testes (Fig. 6A). Although this protein was significantly larger than the predicted size for Comr (68 kDa) the low pI of the protein

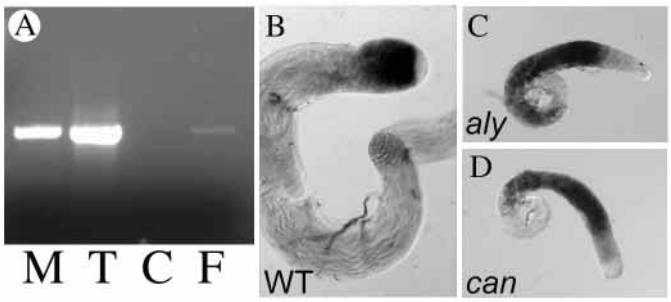

Fig. 5. *comr* is expressed most highly in early primary spermatocytes. (A) RT-PCR of *comr* from wild-type whole males (M), testis (T), a male carcass lacking testis (C) and whole females (F). In males, the transcript was testis specific. Very low levels of transcript were detected in the adult female sample. (B-D) RNA in situ hybridisation using a *comr* probe revealed the transcript was most abundant in early primary spermatocytes in wild type (B). Transcription of *comr* was unaffected in *aly5* (C) or *can*^{3} (D) mutant testes.

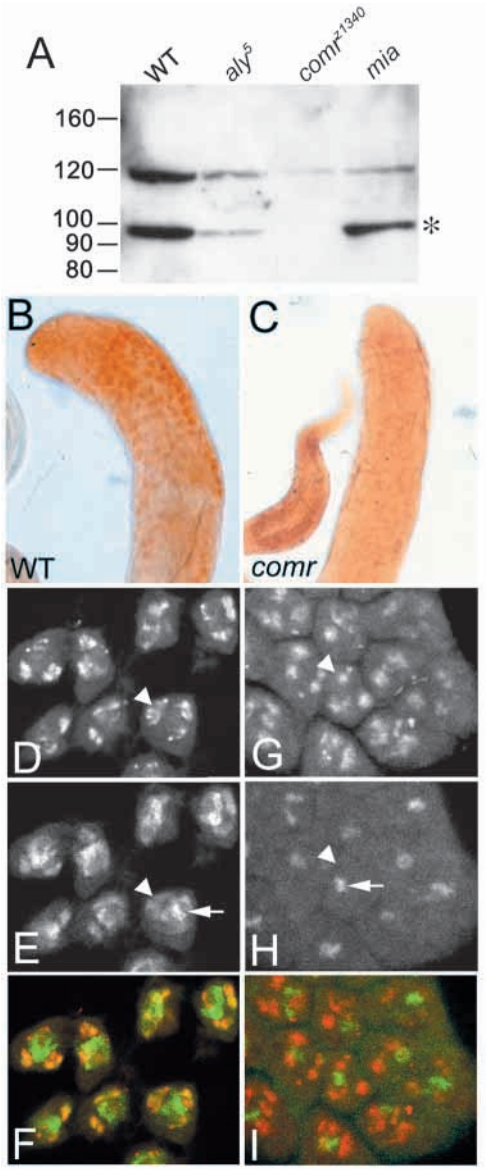

Fig. 6. Comr protein is nuclear in primary spermatocytes. (A) Western blot with the anti-Comr peptide antibody against testis extracts from wild-type (WT), *aly*^{5}, *comr*^{z1340} and *mia* testes. The anti-Comr antibody recognised two bands, one at just under 100 kDa, the other at 120 kDa. The 120 kDa antigen was detected in all the samples, whereas the 100 kDa antigen (indicated by an asterisk) was absent from *comr*^{z1340} mutant testes. This corresponds to Comr protein. (B,C) Immunohistochemistry using the anti-Comr antibody revealed a spotty localisation in the wild-type testes, which corresponds to nuclear staining of the primary spermatocytes. Only background nonspecific staining of *comr*^{z1340} could be detected under these conditions. (D-I) Fluorescence staining of anti-Comr (E,H; green in merged images) and DNA (D,G; red in merged images). In wild-type primary spermatocytes, Comr protein was found throughout the nucleus, but was concentrated in several distinct domains (E). The arrowhead indicates specific Comr staining, overlapping the regions of highest DNA staining intensity. The arrow indicates bright staining attributed to the crossreacting band, not associated with strong DNA staining. *comr*^{z1340} mutant spermatocytes had much lower intensity staining in the chromosomal regions with the anti-Comr antibody (H, arrowhead). Residual staining was restricted to a small region in each nucleus not associated with DNA (H, arrow).

could affect its mobility in SDS-PAGE. We expressed Comr with an N-terminal Flag tag in mammalian tissue culture cells. Western blot analysis of these transiently transfected cells using anti-flag antibodies and the anti Comr antibody showed that ectopically expressed tagged Comr protein migrated as a single band with an apparent molecular weight of 100 kDa (data not shown). The anti-Comr antibody also crossreacts with a 120 kDa protein present in wild type, *Df(2R)Egfr3/Df(2R)X58-7* and *comr*^{Z1340} mutant testes (upper band in Fig. 6A).

In wild-type testes the anti-Comr antibody recognised the nuclei of young and maturing primary spermatocytes (Fig. 6B). Whole-mount immunohistochemistry revealed staining throughout the primary spermatocytes nuclei, as well as a single more concentrated spot of staining within each nucleus. No staining was detected in cells undergoing the meiotic divisions, or at any later stage. When *comr*^{Z1340} mutant testes were stained under the same conditions, the antibody still recognised the single spot within each nucleus, but the general nuclear staining was absent (Fig. 6C). Therefore Comr protein appeared to localise throughout the nucleus in primary spermatocytes. The concentrated spot in the nucleus is likely to be due to crossreactivity of the antibody to the 120 kDa protein.

To explore the relationship between Comr localisation and chromatin in primary spermatocytes, we carried out indirect immunofluorescent staining of Comr double labelled with the DNA dye propidium iodide. Staining with the anti-Comr antibody was restricted to the nuclei of primary spermatocytes. Meiotic and post-meiotic cells did not stain with the antibody. Additionally no staining of any somatic cell type (e.g. cyst cells, sheath or accessory gland) was observed, showing that the protein is germ cell specific. Within primary spermatocyte nuclei the anti-Comr antibody showed two distinct types of staining pattern. A brightly stained ‘stringy’ region (arrow in Fig. 6E) probably corresponds to the darkly stained spot seen in the immunohistochemistry. This may be the Y-loops, a very decondensed region of the Y chromosome, which is transcribed in primary spermatocytes. Weaker, somewhat spotty, staining was found throughout the nucleus, concentrated near the condensed regions of chromatin (arrowheads in Fig. 6D,E). Again, to determine which component(s) of this pattern were attributable to the crossreacting antigen, we stained *comr*^{Z1340} mutant testes. The stringy staining in a small region of the nucleus was still detected in *comr* mutant spermatocytes (Fig. 6H, arrow). However the general nuclear staining was absent from these cells (Fig. 6G,H, arrowheads), confirming our findings from the immunohistochemistry. We conclude that Comr protein is expressed only in primary spermatocytes in the testis, that it is nuclear and that is associated with regions of chromatin.

The nuclear localisations of Comr and Aly are mutually dependent

The identical mutant phenotypes of *comr* and *aly* suggest that they act in the same pathway; we were interested in examining whether *comr* regulated *aly* or vice versa. To determine how

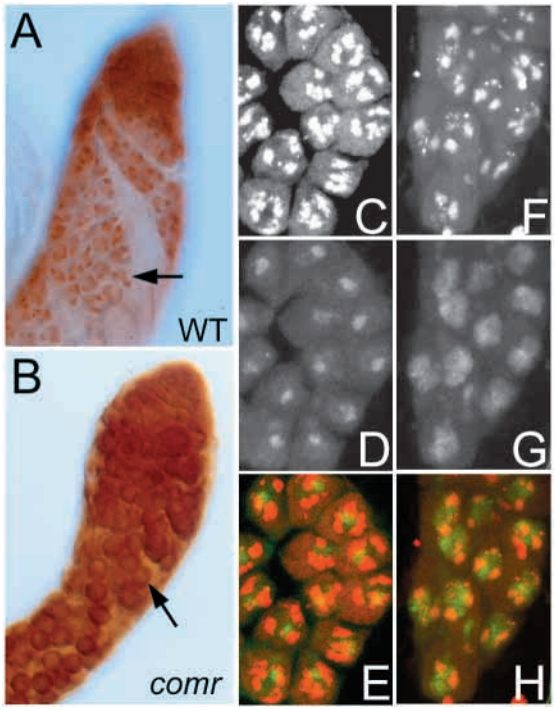

Fig. 7. The nuclear localisations of Aly and Comr are mutually dependent. (A,B) Immunohistochemistry using an anti-Aly antibody. In wild-type (A) testes, Aly protein localised to the nuclei of maturing primary spermatocytes (arrow). In *comr*^{z1340} mutant testes (B) the staining of primary spermatocytes was exclusively cytoplasmic (arrow). (C-H) Fluorescence staining with anti-Comr (D,G; green in merge) and DNA (C,F; red in merge). In *aly*^{5} mutant spermatocytes (C-E) Comr protein was found only in one spot in each nucleus. In *mia* mutant spermatocytes, Comr protein was found throughout the nucleus (F-H).

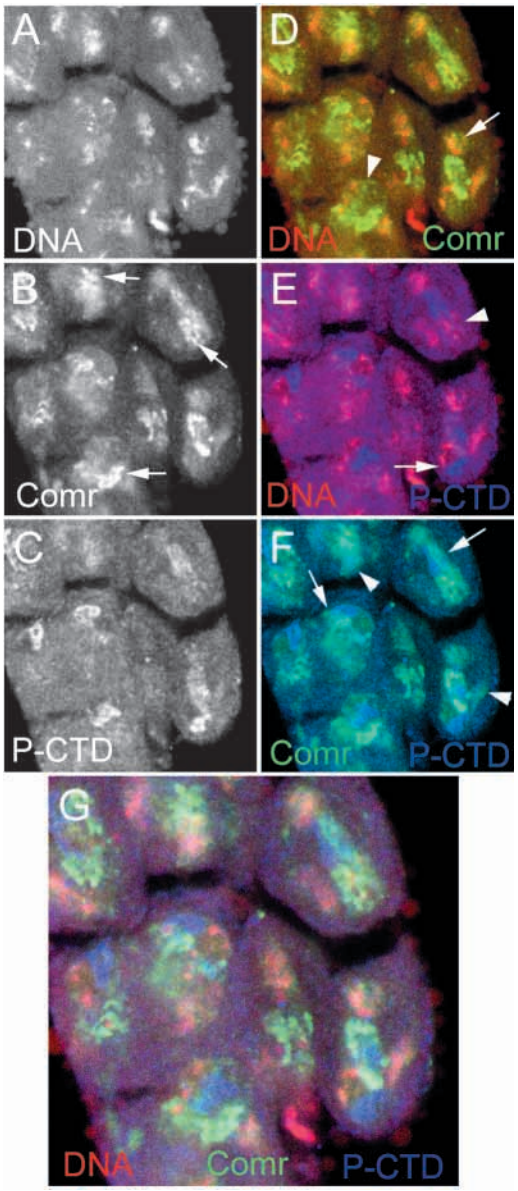

Fig. 8. mRNA transcription is associated with a subset of the Comr-positive chromatin. Triple labelling of DNA (A; red in merged images, D,E,G), Comr (B; green in merged images, D,F,G) and P-CTD (C; blue in merged images, E-G) in part of a cyst of wild-type primary spermatocytes. The nonspecific staining of the anti-Comr antibody is indicated by arrows in B. (D) Most but not all Comr staining associates with DNA. Arrowhead in D indicates a region of anti-Comr staining that does not colocalise with strong DNA staining. The arrow in D indicates a region of colocalisation of Comr with DNA. (E) Strong P-CTD reactivity was not associated with strong DNA staining. The arrowhead in E denotes a chromatin domain that is adjacent to, but not co-localised with, P-CTD reactivity. The region indicated by the arrowhead shows a region of DNA staining accompanied by weak P-CTD staining. (F) Comr staining was present in regions of mRNA transcription. Some regions of high transcription also had high Comr staining (arrows), other transcriptionally active regions had weaker Comr staining (arrowheads).

comr interacts with known meiotic arrest loci, we first investigated the expression of *comr* and *aly* in various mutant backgrounds. *comr* transcript was detected by in situ hybridisation and RT-PCT at a level similar to wild type in *aly* mutant testes (Fig. 5C, and data not shown). Transcription of *comr* was also detected in testes of *can*^{3} (Fig. 5D). Comr protein was detected by western blotting in testis extracts from *aly* mutant males, as well as in testis extracts from males mutant for the *can*-class meiotic arrest gene, *mia* (Fig. 6A). Therefore, transcription and translation of *comr* is upstream of the action of any known meiotic arrest mutant. The level of Comr protein in *aly* mutant testes was lower than in *mia* mutant testes, suggesting a potential role for *aly* in ensuring the accumulation of Comr protein to normal levels. The crossreacting 120 kDa antigen also appeared to be less abundant in all the mutant genotypes compared with wild type. *aly* transcript and protein levels were similar to wild type in *comr*^{Z1340} mutant testes (data not shown).

The similar localisation pattern of Comr and Aly proteins, combined with their identical mutant phenotypes, suggested that the proteins may interact directly or indirectly. To dissect this relationship, we examined the subcellular localisation pattern of one protein in a background mutant for the other. As both *aly* and *comr* are expected to act upstream of the *can*-class genes, staining of *mia* mutant testes was used to control for nonspecific effects of the developmental arrest characteristic of all the meiotic arrest mutants. In wild-type testes Aly protein was localised to the nuclei of maturing primary spermatocytes

(Fig. 7A). Nuclear staining of primary spermatocytes in cysts gave a spotty appearance to the apical region of the testis. By contrast, Aly protein showed a honeycomb distribution pattern in *comr*^{Z1340} mutant spermatocytes (Fig. 7B). The holes in this pattern correspond to the nuclei; therefore, Aly protein failed to translocate to the nucleus and instead remained cytoplasmic in *comr*^{Z1340} mutant spermatocytes. This indicates that the nuclear localisation of Aly protein is dependent on *comr* function.

The reciprocal experiment revealed that Comr protein localisation depended on normal function of *aly*. We carried out immunofluorescence with anti-Comr antibody on *aly*^{5} and *mia* mutant testes. The anti-Comr staining of *aly*^{5} and *comr*^{Z1340} mutant primary spermatocytes was indistinguishable. A single stained spot, corresponding to the 120 kDa antigen was present in the mutant cells (Fig. 7D). *mia* mutant spermatocytes showed staining throughout the nucleus, in addition to the bright spot (Fig. 7G). By immunohistochemistry, the anti-Comr antibody staining of *aly*^{5} and *comr*^{Z1340} mutant spermatocytes were indistinguishable (data not shown), with only one small region of the nucleus stained. *mia* and *can*^{3} testes probed with the anti Comr antibody had staining throughout the nuclei of primary spermatocytes, in addition to the single strongly stained spot (data not shown). In both immunostaining techniques, the staining intensity of the spot in the nucleus was less than in wild type, consistent with the reduction in level of the 120 kDa antigen seen in the western blotting. Thus, the nuclear localisation of Comr depended on *aly*, but did not depend on the normal function of the can-class meiotic arrest genes *can* and *mia*.

Relationship of Comr to transcription

comr mutant spermatocytes are defective for transcription of a number of cell cycle and spermatid differentiation genes (Fig. 2). To investigate how Comr protein localisation in the nucleus is associated with mRNA transcription, we used an antibody specific to active RNA polymerase II phospho-C-terminal domain (P-CTD) in triple labelling experiments. Fig. 8 shows the relationship between Comr, DNA and active transcription in primary spermatocytes. Staining with anti P-CTD revealed that the regions of the nucleus with most active transcription were adjacent to, but not overlapping with, regions of visible (i.e. condensed) DNA (arrow in Fig. 8D). The active transcription was found in domains within the nucleus, not randomly distributed. Lower levels of detectable P-CTD colocalised with the more condensed DNA (arrowhead in Fig. 8D). The highest levels of active transcription partially overlapped with high levels of Comr protein (arrows in Fig. 8F), although some regions of strong P-CTD staining were associated with weaker Comr staining. All of the regions where transcriptional activity was detected had at least some Comr protein present (arrow in Fig. 8F), although P-CTD staining was not found in all regions containing Comr protein. Thus, Comr protein was not exclusively localised on the pol II-transcriptionally active chromatin, but all the chromatin where RNA polymerase II is transcriptionally active had at least some associated Comr protein.

DISCUSSION

comr is the second *aly*-class meiotic arrest gene

In spermatogenesis, a major transition occurs as the mitotically amplifying population of spermatogonia cease mitosis and develop into primary spermatocytes. These primary spermatocytes become committed to undergoing the meiotic divisions, and then differentiating into spermatozoa. This change in cell behaviour is associated with a dramatic switch in the transcript profile: some genes are downregulated and many are upregulated or switched on for the first time. The meiotic arrest genes of *Drosophila* are crucial for regulating transcription in primary spermatocytes. Numerous genes require the activity of the meiotic arrest genes for their transcription. Earlier analysis showed that *aly* is different from the other meiotic arrest genes *can*, *mia* and *sa*, in that it is required for the transcriptional activation of more target genes, and for normal chromosome structure. Based on this, we proposed that *aly* acts upstream of the *can*-class genes in primary spermatocytes. We have shown that *comr* is a novel meiotic arrest gene, that *comr* mutant spermatocytes fail to transcribe *twine* and *mst87F* as well as many other target genes, and that the cells arrest with abnormal chromatin morphology. This demonstration that the *comr* mutant phenotype is indistinguishable from that of *aly* supports the segregation of the meiotic arrest genes into *aly* and *can* classes. The chromosome morphology defect seen in both *aly* and *comr* mutant lines suggests that the pathway in which they act has a role in the maintenance of normal chromatin structure.

comr and *aly* are mutually dependent

Comr and Aly are mutually dependent: Aly protein requires *comr* for its normal nuclear localisation, and Comr protein requires *aly* for its normal nuclear localisation. Thus, the phenotypes of the two mutants are identical, because ablation of one essentially knocks out the other by preventing its normal localisation. It seems likely that Aly and Comr proteins physically interact or are both essential components of a multi-subunit complex. Anchoring of either protein in the nucleus would then be dependent on the formation of this complex, loss of either would give the aberrant protein localisation patterns seen. To date we have been unable to detect any direct physical interaction between Aly and Comr proteins (J. J. and H. W.-C., unpublished), so we would favour a hypothesis where Aly and Comr are in a complex with at least one other protein. Aly nuclear localisation is regulated; this regulation need not be solely dependent on Comr. For example, cytoplasmic Aly could be phosphorylated, and dephosphorylation could allow nuclear entry and therefore interaction with Comr. This protein complex would then be able to attach to chromatin.

aly, *comr* and *can* transcripts are all transcribed in a characteristic pattern in wild-type testes (Hiller et al., 2001; White-Cooper et al., 2000). High levels of transcripts are found in early primary spermatocytes, and the levels decrease as the spermatocytes mature. None of the transcripts are detectable as the cells enter the meiotic divisions. This pattern is entirely consistent with the role of these genes in activation of transcription of many new genes in early primary spermatocytes, and the continued high transcriptional activity throughout primary spermatocytes development.

It is interesting to note that both *comr* and *aly* transcripts appear to be expressed at low levels in tissues other than the testis. We were able to detect some *comr* transcript by RT-PCR in females and in all stages of embryos examined (0-1, 3-5 and 12-24 hours). *aly* mRNA was also detected at low levels in the older embryo samples (J. J. and H. W.-C., unpublished).

However null mutants of both *comr* and *aly* are fully viable and female fertile, showing that the expression of these genes outside the testis is not essential. It is possible that *comr* and *aly* have a redundant function in these other tissues. A second *Drosophila* homologue of *aly* has been identified (White-Cooper et al., 2000), but there is no obvious *comr* homologue in the genome. It is attractive to postulate that the meiotic arrest genes are all under the control of the same transcription factor, so that they are all activated early in the primary spermatocyte programme of development.

How do *aly* and *comr* regulate transcription?

The predicted *comr* protein is novel. While its predicted size is 68 kDa, Comr protein (from testes or expressed in mammalian tissue culture cells) migrates at about 100 kDa in SDS-PAGE. This aberrant mobility on SDS-PAGE gels may be due to the acidity of the protein retarding its migration. The low predicted pI of the protein may provide some clues as to its biochemical function. The protein is rather acidic throughout its length, as well as having a very acidic region near the C terminus. In this regard, it bears some similarity to the acidic histone chaperone protein nucleoplasmin, which is important for nucleosome assembly and remodelling during transcription (Chen et al., 1994; Earnshaw et al., 1980). It is possible that the acidic domain on Comr interacts with the basic histone proteins to alter chromatin structure.

aly encodes a homologue of the *C. elegans* SynMuvB gene *lin-9* (Beitel et al., 2000; White-Cooper et al., 2000). The SynMuvA and B genes act in two genetically redundant pathways to repress vulval cell fate and promote hypodermal cell fate in the vulval precursor cells (Chen and Han, 2001; Fay and Han, 2000; Ferguson and Horvitz, 1989). The SynMuvB genes include subunits of the NURD histone deacetylase/nucleosome remodelling complex, and probably regulate genes involved in vulval formation by altering chromatin structure (Lu and Horvitz, 1998; Solari and Ahringer, 2000). By analogy, *aly* may activate such a NURD complex in primary spermatocytes. *comr* could act with *aly* as a regulator of the complex. Alternatively *comr* and maybe also *aly* could function as testis specific components of the NURD complex. In this model Comr (and Aly) proteins would be directly involved in nucleosome remodelling, Comr protein perhaps interacting with histones as postulated above.

The predicted Comr protein did not contain any sequence motifs with known DNA-binding activity. Nevertheless, Comr protein is found in cells in close association with the chromatin, suggesting that the chromatin localisation of Comr may be mediated by protein-protein, rather than protein-DNA, interactions. A candidate region for mediating such protein-protein interactions is the PB-1-like motif. This region of Comr is not similar enough to the PB-1 consensus to score a significant match, therefore it is unlikely that the domain is a true PB-1 domain, interacting with the PC motif. However this PB-1 like region of Comr could be responsible for mediating protein-protein interactions by binding to a motif similar to PC.

It has recently been shown that the meiotic arrest gene *can* encodes a testis-specific homologue of dTAF80, a subunit of the basal transcription factor $TF_{II}D$ (Hiller et al., 2001). $TF_{II}D$ consists of TATA-binding protein and associated factors, binds to the promoter region, interacts with transcriptional activator proteins and helps in recruitment of the RNA polymerase II holoenzyme complex to the transcription initiation site (Dynlacht et al., 1991; Goodrich et al., 1996; Roeder, 1996). The human and yeast homologues of *can* are also found in the histone acetyl-transferase (HAT) complex PCAF or SAGA (Grant et al., 1998; Ogryzko et al., 1998). As *can* is not required for all transcriptional activation in primary spermatocytes, it may be that spermatocytes have two $TF_{II}D$ complexes, each with a different set of target genes. *aly* and *comr* could function by altering the chromatin structure at the site of target promoters so that the testis specific, Can-containing, $TF_{II}D$ complex can bind and activate transcription. This would make all the *can*-dependent transcripts also dependent on *comr* and *aly*, consistent with our observations. However this simple model cannot explain why some genes, namely *cyclinB*, *twine* and *boule,* require *aly* and *comr* but not the *can*-class genes for their expression. These *aly*-class-dependent genes are all required for normal meiotic cell cycle progression in testes; however, they are not transcribed exclusively in primary spermatocytes. *cyclinB* is required for mitosis, so is expressed throughout development (Lehner and O'Farrell, 1990), *twine* is required for meiosis in the female germline so is also expressed in ovaries (Alphey et al., 1992), and *boule* transcripts have been found in cDNA libraries derived from heads (H. W.-C., unpublished) (Rubin et al., 2000). Their transcription in primary spermatocytes may depend on particular chromatin structure to facilitate binding of a spermatocyte specific transcription factor, which would act in conjunction with the conventional $TF_{II}D$ complex. This, or a related, postulated specific transcription factor could also be required for transcription of *can*-class dependent genes by interacting with the testis specific $TF_{II}D$ complex. Fig. 9 shows a model for how target promoters could be regulated, first by chromatin remodelling promoted by Aly and Comr, then by transcription factor binding and recruitment of $TF_{II}D$ complexes.

How does the pathway interact with the transcriptional pattern

When assessed using immunostaining, both Comr and Aly proteins persist until the G2-M transition of meiosis I, but become undetectable as the chromosomes condense in pro-metaphase I. At this point, transcriptional activity is shut down. The cause and effect relationship between transcription shut down and Aly/Comr disappearance events not clear. Perhaps transcription shuts down because Comr and Aly are degraded in response to the same cues that signal chromosome condensation. Alternatively, the proteins could become physically excluded from the DNA during chromosome condensation, and then degraded.

Transcription from a particular promoter can be viewed as a cycle of polymerase binding, initiation of transcription, promoter clearance and termination. During this cycle, the phosphorylation state of the CTD of RNA polymerase II changes. Unphosphorylated pol II is competent to enter the pre-initiation complex, as promoter clearing occurs the pol II becomes multiply phosphorylated on the CTD (Conaway et al., 2000; Dahmus, 1996). At termination of transcription, RNA polymerase II is dephosphorylated and released, ready for another cycle. Hence, antibodies specific to phosphorylated CTD only label the pool of RNA polymerase II that is actively transcribing. The antibody used in this study (H5) specifically

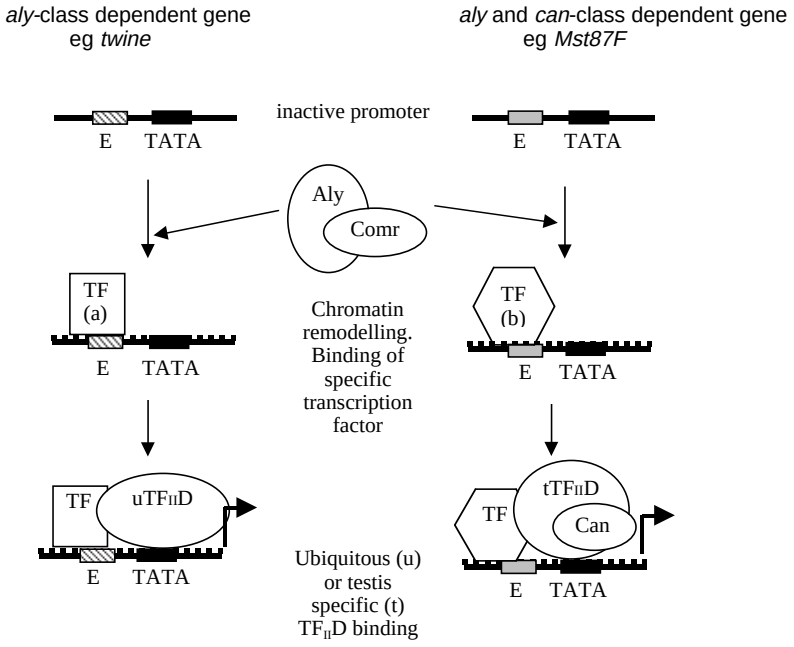

Fig. 9. Model for meiotic arrest gene product action at target promoters. Target promoters may be dependent solely on the function of the *aly*-class genes, including *aly* and *comr* (e.g. *twine*), or they could additionally depend on the *can*-class genes (e.g. *Mst87F*). The promoter regions of these genes differ, here denoted by shaded or hatched enhancer elements (E) 5′ of the TATA box. The *aly*-class proteins Aly and Comr would work together to promote local chromatin remodelling (small boxes on DNA), which would allow binding of specific transcription factors [TF(a) or TF(b)] to the enhancer elements. Binding of these transcription factors allows transcriptional activation by recruitment of the ubiquitous (u) $TF_{II}D$ in the case of *can*-independent genes, or testis specific (t) $TF_{II}D$, including Can, in the case of *can*-class dependent genes.

recognises phosphorylation of Ser2 in the heptapeptide CTD repeat (Patturajan et al., 1998).

We have demonstrated that the most active regions of mRNA transcription are not associated with strong DNA staining. Lower levels of transcriptional activity were found to be associated with this DNA. This is expected, as we would predict that active transcription occurs on the most decondensed, and therefore weakly staining, regions of DNA. The staining clearly shows that there are discrete domains within the nucleus with high levels of transcriptional activity, rather than the active regions of chromatin being randomly distributed within the nucleoplasm. Comr protein colocalised with DNA staining, and therefore it is not surprising that we found Comr protein associated with all regions of transcriptional activity. There was no clear correlation between the intensity of Comr staining and the presence or absence of P-CTD immunoreactivity. Within each nucleus some regions of high mRNA transcription were associated with high Comr, other regions were associated with low Comr levels. Not all Comr-positive regions of chromatin were transcriptionally active. Given this distribution how does Comr control the transcription in primary spermatocytes of some genes but not others? Comr protein seems to be present on more regions of chromatin than those that are actively transcribing, so it is unlikely that binding of Comr to chromatin regions is solely responsible for marking them for transcriptional activation. The Comr-containing complex must interact with another factor to give the promoter specificity we observe. For many promoters, that other factor may be the Can-containing $TF_{II}D$ complex; the other factor for can-independent promoters remains unidentified. Because neither of the *aly*-class genes described to date encode direct DNA-binding proteins, it is likely that more genes of this class, which encode DNA-binding transcription factors, remain to be identified. These would be likely to contribute also to the promoter specificity of the meiotic arrest transcriptional activation pathway through sequence-specific DNA binding.

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REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A. and Gocayne, J. D., Amantitides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F. et. al. (2000). The genome sequence of Drosophila melanogaster. *Science* **287**, 2185-2195.

Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P. and Glover, D. M. (1992). *twine*, a *cdc25* homologue that functions in the male and female germlines of *Drosophila*. *Cell* **69**, 977-988.

Beitel, G. J., Lambie, E. J. and Horvitz, H. R. (2000). The *C. elegans* gene *lin-9*, which acts in an Rb-related pathway, is required for gonadal sheath cell development and encodes a novel protein. *Gene* **254**, 253–263.

Chen, H., Li, B. and Workman, J. (1994). A histone-binding protein, nucleoplasmin, stimulates transcription factor binding to nucleosomes and factor-induced nucleosome disassembly. *EMBO J.* **13**, 380-390.

Chen, Z. and Han, M. (2001). C. elegans Rb, NuRD, and Ras regulate lin-39-mediated cell fusion during vulval fate specification. *Curr. Biol.* **11**, 1874-1879.

Conaway, J. W., Shilatifard, A., Dvir, A. and Conaway, R. C. (2000). Control of elongation by RNA polymerase II. *Trends Biochem Sci.* **25**, 375-380.

Dahmus, M. (1996). Reversible phosphorylation of the C-terminal domain of RNA polymerase II. *J. Biol. Chem.* **271**, 19009-19012.

Dynlacht, B. D., Hoey, T. and Tjian, R. (1991). Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **66**, 563-576.

Earnshaw, W. C., Honda, B. M., Laskey, R. A. and Thomas, J. O. (1980). Assembly of nucleosomes: the reaction involving X. laevis nucleoplasmin. *Cell* **21**, 373-383.

Fay, D. S. and Han, M. (2000). The synthetic multivulval genes of C-elegans: Functional redundancy, Ras-antagonism, and cell fate determination. *Genesis* **26**, 279-284.

Ferguson, E. L. and Horvitz, H. R. (1989). The multivulva phenotype of certain *Caenorabditis elegans* mutants results from defects in two functionally redundant pathways. *Genetics* **123**, 109-121.

FlyBase (1999). The FlyBase database of the Drosophila genome projects and community literature. *Nucleic Acids Res.* **27**, 85-88.

Fuller, M. T. (1993). Spermatogenesis. In *The Development of Drosophila*, Vol. 1 (ed. M. Bate and A. Martinez-Arias), pp. 71-147. Cold Spring Harbor, New York: Cold Spring Harbor Press.

Goodrich, J., Cutler, G. and Tjian, R. (1996). Contacts in context: promoter specificity and macromolecular interactions in transcription. *Cell* **84**, 825-830.

Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R. r. and Workman, J. L. (1998). A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* **94**, 45-53.

Hales, K. G. and Fuller, M. T. (1997). Developmentally regulated mitochondrial fusion mediated by a conserved novel predicted GTPase. *Cell* **90**, 121-129.

Hiller, M. A., Lin, T.-Y., Wood, C. and Fuller, M. T. (2001). Developmental regulation of transcription by a tissue-specific TAF homolog. *Genes Dev.* **15**, 1021-1030.

Ito, T., Matsui, Y., Ago, T., Ota, K. and Sumimoto, H. (2001). Novel modular domain PB1 recognizes PC motif to mediate functional protein-protein interactions. *EMBO J.* **20**, 3938-3946.

Kemphues, K. J., Kaufman, T. C., Raff, R. A. and Raff, E. C. (1982). The testis-specific β-tubulin subunit in Drosophila melanogaster has multiple functions in spermatogenesis. *Cell* **31**, 655-670.

Kerrebrock, A. W., Moore, D. P., Wu, J. S. and Orr-Weaver, T. L. (1995). mei-S332, a Drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell* **83**, 247-256.

Lehner, C. and O'Farrell, P. H. (1990). The roles of Drosophila cyclins A and B in mitotic control. *Cell* **61**, 535-547.

Lin, T.-Y., Viswanathan, S., Wood, C., Wilson, P. G., Wolf, N. and Fuller, M. T. (1996). Coordinate developmental control of the meiotic cell cycle and spermatid differentiation in *Drosophila* males. *Development* **122**, 1331-1341.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. New York: Academic Press.

Lu, X. W. and Horvitz, H. R. (1998). lin-35 and lin-53, two genes that antagonize a C-elegans Ras pathway, encode proteins similar to Rb and its binding protein RbAp48. *Cell* **95**, 981-991.

Ogryzko, V., Kotani, T., Xiaolong, Z., Schiltz, R. L., Howard, T., Qin, J. and Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94**, 35-44.

Olivieri, G. and Olivieri, A. (1965). Autoradiographic study of nucleic acid synthesis during spermatogenesis in *Drosophila melanogaster*. *Mutat. Res.* **2**, 366-380.

Patturajan, M., Schulte, R. J., Sefton, B. M., Berezney, R., Vincent, M., Bensaude, O., Warren, S. L. and Corden, J. L. (1998). Growth-related changes in phosphorylation of yeast RNA polymerase II. *J. Biol. Chem.* **273**, 4689-4694.

Peschon, J. J., Behringer, R. R., Brinster, R. L. and Palmiter, R. D. (1987). Spermatid-specific expression of protamine 1 in transgenic mice. *Proc. Natl. Acad. Sci. USA* **84**, 5316-5319.

Ponting, C. P., Ito, T., Moscat, J., Diaz-Meco, M. T., Inagaki, F. and Sumimoto, H. (2002). OPR, PC and AID: all in the PB1 family. *Trends Biochem Sci.* **27**, 10.

Price, J. V., Clifford, R. J. and Schupbach, T. (1989). The maternal ventralizing locus torpedo is allelic to faint little ball, an embryonic lethal, and encodes the Drosophila EGF receptor homolog. *Cell* **56**, 1085-1092.

Roeder, R. G. (1996). The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem. Sci.* **21**, 327-335.

Rubin, G. M., Yandell, M. D., Wortman, J. R., Miklos, G. L. G. and Nelson, C. R., Hariharan, I. K., Fortini, M. E., Li, P. W., Apweiler, R., Fleischmann, W. et. al. (2000). Comparative genomics of the eukaryotes. *Science* **287**, 2204-2215.

Schulz, R. A., Miksch, J. L., Xie, X. L., Cornish, J. A. and Galewsky, S. (1990). Expression of the Drosophila *gonadal* gene: alternative promoters control the germ-line expression of monocistronic and bicistronic gene transcripts. *Development* **108**, 613-622.

Solari, F. and Ahringer, J. (2000). NURD-complex genes antagonise Ras-inducted vulval development in *Caenorhabditis elegans*. *Curr. Biol.* **10**, 223-226.

Wakimoto, B. T. (2000). Doubling the rewards: testis ESTs for Drosophila gene discovery and spermatogenesis expression profile analysis. *Genome Res.* **10**, 1841-1842.

White-Cooper, H., Schafer, M. A., Alphey, L. S. and Fuller, M. T. (1998). Transcriptional and post-transcriptional control mechanisms coordinate the onset of spermatid differentiation with meiosis I in *Drosophila*. *Development* **125**, 125-134.

White-Cooper, H., Leroy, D., MacQueen, A. and Fuller, M. T. (2000). Transcription of meiotic cell cycle and terminal differentiation genes depends on a conserved chromatin associated protein, whose nuclear localisation is regulated. *Development* **127**, 5463-5473.

Zhou, Z., Luo, M., Straesser, K., Katahira, J., Hurt, E. and Reed, R. (2000). The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* **407**, 401-405.