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1. The effect of a transposition of part of the *abdominal-B* component of the bithorax complex upon segment identity in *Drosophila*

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The bithorax complex contains a number of interdependent genetic elements involved in the cell-heritable specification of differences in segment identity during formation of the larval and imaginal thorax and abdomen. In this complex the right-hand of the three complementation groups of lethal mutations, *Abdominal-B* (*Abd-B*), defines a set of functions required only in posterior abdominal metameres of the insect body. We have used an irradiation-induced chromosomal transposition with a break-point within *AbdB* to see whether the two complementary fragments carry independent functions within *ABdB*.

The transposition $Tp(3, Y)AbdB^{S10}$ can be separated into two deficiencies, one of which is the left-hand majority part of the bithorax complex ($Df(3R)AbdB^{S10}$). A deletion for the right hand minority part of the complex is produced by combining the duplication $Dp(Y)AbdB^{S10}$ with a deletion for the entire complex. Both these deficiency genotypes are embryonic lethals and the detailed morphological features of each segment have allowed us to define the relationships of genes left and right of the transposition break point.

2. Functional analysis of the transcriptional control region of the *Drosophila melanogaster* transposable element *copia*

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Introduction of *copia*-based vectors into *D. hydei* cells results in their high level expression with transformed cell lines containing multiple copies of vector, apparently stably integrated into host genomic DNA. Using transformation frequency and transient expression analysis as an assay of promoter strength, we have defined regions of *copia* essential for expression. We find that essential sequences reside within the long terminal repeat (LTR), but 3′ to the site of initiation of *copia* RNA. Deletion of the SV40 enhancer-like sequences from *copia* appears to have no effect on expression.

3. Recent amplification of a P factor deletion-derivative in European populations of *Drosophila melanogaster*

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Complete 2·9 kbp P factors cause hybrid dysgenesis in *D. melanogaster* when they are in an M cytotype background (i.e. not repressed). P factor deletion-derivatives are observed in all natural populations, each generally being present in single or few copies only. By contrast, European populations contain 20–30 copies per haploid genome of a 1.15 kbp P element. This element is completely absent in African and North American populations. Sequence analysis of cloned representatives of these elements shows a 100 per cent homology with American P factors from the $\pi 2$ strain. Additionally, the data throw light on the mechanism of deletion formation and the repression of P element

transposition. Deletion formation may be occurring either via slippage replication during transposition or recombination in extra-chromosomal elements, involving very short direct repeat DNA sequences. Deletion-derivatives have been invoked in the repression of P element transposition. The successful and recent spread of the 1·15 element into European population indicates the manner in which this repression might take effect.

4. A transposable element is associated with *Om* mutations in *Drosophila ananassae*

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Om mutability in *Drosophila ananassae* involves spontaneous mutation at some 20 loci, resulting in semidominant, non-pleiotrophic eye morphology defects (Hinton, Genetics, 106, 631). *Om(1D)9*, a particularly unstable *X*-linked *Om* allele, produced several derivatives, one of which, *Om(1D)9g*, has a more extreme *Om* phenotype and was accompanied by a singed bristle mutant, Sn^{9g}. DNA probes from the *sn* locus of *D. melanogaster* were used to clone the homologous region of *D. ananassae*. Analysis of sn^{9g} DNA detected a 6·5 kb insert. Genomic Southern blotting and *in situ* hybridisation techniques indicated that this insert is repetitive and dispersed. Genetic mapping was used to establish that there is homology between the 6·5 kb sn^{9g} insert and *Om* mutants at four *X*-linked loci. One of the *X*-linked *Om* loci, *Om(1D)*, has been cloned from *Om(1D)9g* and two copies of a copia-like transposable element are present.

5. Structure and origin of *Copia* extrachromosomal elements

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The *Drosophila* transposable element *copia* is closely related to retroviruses. We have studied extrachromosomal *copias* from *Drosophila* cell cultures to determine whether they derive from reverse transcription, semi-conservative replication or genomic excision, with the following results: (1) BUdR density labelling experiments show a large proportion of linear extrachromosomal *copias* are derived non-semiconservatively using an aphidicholin-resistant DNA polymerase. (2) At least 50 per cent of 1LTR- and 2LTR circular *copias* are derived semiconservatively although their synthesis is somewhat aphidicholin sensitive. (3) A significant proportion (>10 per cent) of newly-synthesised *copia* circles are derived semiconservatively and their synthesis is aphidicholin-sensitive. Therefore while the majority of *copia* circles appears to derive from reverse transcription via linear intermediates, a significant proportion appear to be replicating semiconservatively. Furthermore, the LTR-LTR junction sequence of cloned 2LTR *copia* circles is inconsistent with their derivation by *copia* linear circularisation; indeed two of them are most easily explained by a genomic excision model. We have cloned linear extrachromosomal *copias* and sequenced the ends of four clones. Three of the molecules have flush ends identical to the ends of integrated *copias*, one has a single nucleotide deletion at one end. None of these molecules possesses the ends demanded by a simple tRNA-primed reverse transcriptional model. We suggest from these data that extrachromosomal *copias* are transposition intermediates which may be derived in three different ways, namely (1) Reverse transcription from *copia* RNA by a similar but not identical mechanism to retroviruses. (2) Semiconservative replication as plasmids. (3) Excision from the *Drosophila* genome. If extrachromosomal *copias* are integrative precursors then the two latter mechanisms imply that *copia* may transpose (albeit rarely) entirely via DNA intermediates. This supports the hypothesis that retroviruses may have evolved from DNA transposons.

6. Comparison of the genetic behaviour of the Tam 3 element at two pigment loci in *Antirrhinum majus*

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Instability of the $pallida^{recurrens}$ (pal^{rec}) allele in *Antirrhinum majus* has been studied for many years and has recently been proved to be due to a transposable element Tam 3. Through an extensive

crossing programme this element was transferred to the unlinked *nivea* locus, a gene encoding chalcone synthase, giving a completely new *nivea*^{recurrens} plant (*niv*^{rec}:Tam3). This new unstable plant has enabled a direct comparison of the Tam 3 element at two distinct genetic loci to be made. Excision rates of Tam 3 from both *pal*^{rec} and *niv*^{rec} plants have been shown to vary in a similar way in response to temperature: plants grown at 15°C show a much higher rate of excision than those grown at 25°C. An unlinked semi-dominant *Stabiliser* (*St*) gene also affects the excision rate at both loci, plants of the *St*/*St* genotype in each case showing a very low rate compared to *st*/*st*. A series of new phenotypes and alleles has been produced from *niv*^{rec}:Tam 3. These *nivea* alleles give either: stable phenotypes, some of which show reduction of pigment intensity when compared to wild type, while others show spacial patterning; or unstable phenotypes, showing altered instability patterns. One difference in the behaviour of Tam 3 at the two loci is the production of relatively large numbers of null alleles from *niv*^{rec}:Tam 3 plants whereas a complete null has not so far been recovered from our *pal*^{rec} lines.

7. A sequence rearrangement associated with a transposable element results in a restricted spatial pattern of gene expression

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The *pallida* (*pal*) locus of *Antirrhinum majus* encodes a product essential for flower pigment biosynthesis. An unstable mutation blocking synthesis, *pal*^{rec}, arises from the insertion of the transposable element Tam 3. A number of unstable alleles (states) presenting new patterns of instability have been derived from *pal*^{rec}. Unstable allele *pal-42* (*pal*$^{maculosa-3}$) shows diffuse irregularly distributed spots and sectors of pale red and some discrete spots of wild type intensity. This allele contains an intact Tam 3 element as in *pal*^{rec} but the Tam 3 flanking sequence thought to be 5′ to the *pallida* gene has been replaced by a new sequence. The original flanking sequence is still present in the genome indicative of a rearrangement, possibly an inversion or translocation. Excision of Tam 3 from *pal*-42 has given rise to a stable allele, *pal*-41 (*pal*$^{varicolor-2}$). As a result, the *pallida* gene in allele *pal-41* is placed in a new sequence environment compared to wild type and a new restricted spatial distribution of pigment is observed. The phenotype of *pal-41* appears to result from a quantitative reduction rather than any qualitative alteration of the *pallida* transcript. The different unstable phenotpes of *pal-42* and *pal*^{rec} may simply reflect the different phenotypes which result from Tam 3 excision. In addition, it is possible that the rearrangement may have affected the rate, or timing, of Tam 3 transposition.

8. Genetic activation of the transposable element Tam 2 in *Antirrhinum majus*

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The *nivea* (*niv*) locus of *Antirrhinum majus* encodes the anthocyanin biosynthetic enzyme chalcone synthase. A mutation in the *nivea* locus, *niv*-44, gives rise to white flowers. This mutation is due to the insertion at the first exon-intron boundary of a transposable element, Tam 2, which does not usually transpose. However, crossing *niv*-44 with *niv*-99 produces progeny having white flowers with red spots. It was proposed that the spots were the result of somatic excision of Tam 2. Sequence analysis of a germline revertant allele indicated that imprecise excision of Tam 2 has indeed taken place. Excision of Tam 2 occurs only in *niv*-44/*niv*-99 heterozygotes and not in *niv*-44/*niv*-44 homozygotes, suggesting that the *niv*-99 allele specifically activates Tam 2 transposition. The *niv*-99 allele is a stable derivative of an unstable allele containing another transposable element, Tam 1, in the promoter region. It would therefore seem likely that Tam 1 itself is able to activate transposition of Tam 2.

9. Transposition events in the evolution of interspersed repeated DNA in plants

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A complex pattern of interspersed repeated sequences forms a large proportion of the genomes

of most plants. During the evolution of this interpsersed arrangement, sequences must have been both amplified in number and transposed to new locations. The existence of genetically defined transposable elements in maize and *Antirrhinum* suggests a possible mechanism for this movement. In animals and yeast, transposition via an RNA intermediate appears to have played an important role in the evolution of interspersed repeated DNA.

Interspersed repeats often have sequence structures which are characteristic of the mechanisms by which they became amplified and dispersed. The nature of the interspersion of repeats in the genome of wheat (*Triticum aestivum*) has been thoroughly studied and provides evidence for the transposition of repeated sequences during recent evolution. This paper reports the results of an investigation of some selected interspersed repeated sequences from the wheat genome to look for moleculr structures giving clues to their mode of evolution. Of particular interest is a long repeated sequence which has some structure features which are characteristic of a subset of elements which are transposed via an RNA intermediate. The discovery of this element raises the possibility that this mode of transposition is important in the evolution of the interspersed arrangement of repeated sequences in wheat.

10. The genetic organisation of the yeast transposon, Ty.

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Most of the 30–35 copies of Ty that are dispersed throughout the yeast genome are about 5·9 kb long. They comprise a 5·2 kb unique region, called epsilon, flanked by LTR's of about 340 bp called delta. The major primary transcription product is a "full-length" 5·7 kb RNA that starts and ends in the deltas and has 50 nucleotide terminal repeats. It is therefore structurally analogous to a retroviral genomic RNA. This transcriptional unit is divided into two genes, *TYA* and *TYB*. *TYA* starts at nucleotide 299 and ends at 1619. *TYB* starts with an ACA (threonine) codon (in Ty 1-15) at 1581 and ends close to the end of the epsilon region at 5676. *TYA* and *TYB* overlap therefore by 38 bp and *TYB* is in the +1 reading frame with respect to *TYA*. *TYA* is translated from the 5·7 kb RNA to produce a 50 K protein, p 1. *TYB* is expressed as a 190 kd *TYA*:*TYB* fusion protein, p 3, via a frameshift event that fuses *TYA* and *TYB*. both p 1 and p 3 are proteolytically cleaved to produce mature Ty proteins.

The 5·7 kb RNA is an intermediate in a reverse transcriptase mediated transposition mechanism. Ty RNA, Ty-encoded reverse transcriptase and other Ty proteins are found in Ty-virus like particles (Ty-VLP's) and may function as units of transposition.

11. The effects of P transposable element insertions on expression of the *singed* and *white* loci of *Drosophila melanogaster*

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During P-M hybrid dysgenesis in *Drosophila melanogaster*, a transposase encoded by functional P elements is made in the developing germ cells of the progeny of the dysgenic cross. This catalyses the transposition of both functional and defective P elements, resulting in mutations when the elements insert into or near genes. When such mutants undergo P-M hybrid dysgenesis the elements may exercise either precisely or imprecisely, or partake in complex chromosomal rearrangements. Results from studies of elements inserted into the *singed* and *white* loci, derivatives of these mutations generated in P-M hydrid dysgenesis, and the effects of the insertions on expression of the target gene will be discussed.

12. Novel patterns of plant gene expression arise by transposable element excision and rearrangement

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The *pallida* (*pal*) locus of *Antirrhinum majus* encodes a product essential for flower colour production. One mutation in *pal*, called *pallida*^{recurrens} (*pal*^{rec}) results in flowers with spots or sectors of

pigmentation on an unpigmented background. This mutation is unstable and can give rise to Pal^{+} alleles conferring full red flower colour (revertants) or, more rarely, to *pal* alleles conferring novel quantities or patterns of flower colour. We have shown that the pal^{rec} mutation is due to insertion of a transposable element Tam 3 at the *pal* locus and have used Tam 3 as a probe to clone the *pal* gene. We used this clone to analyse a number of *pal* alleles at the molecular level. The first class of alleles analysed have flowers with levels of stable pigmentation ranging from very pale red to almost full red intensity. These alleles arise by imprecise excision of Tam 3. Alleles with novel spacial distribution of pigment also result from imprecise Tam 3 excision although one allele shows a more complicated rearrangement of the *pal* locus. Alleles which show new patterns of somatic variegation retain all, or part, of the Tam 3 element and can result from small internal Tam 3 deletions or from rearrangement of DNA sequences flanking Tam 3. Imprecise excision and rearrangements is a general property of plant transposable elements and can clearly generate a remarkable range of quantitative and spatial variation in gene expression.

13. Mechanisms of transposition in plants

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In the course of the last 15 years a wealth of evidence on transposition in plants has been gathered from classical genetic experiments, primarily with maize (for reviews see Fincham and Sastry, 1974; Federoff, 1983; Nevers and Saedler, 1977; Nevers *et al.*, 1984). Recently these results have been supplemented by molecular data made available by the cloning and sequencing of several plant transposable elements and by analysis of the pertinent DNA sequences of revertants of transposon induced mutations. The structures of integrated plant transposable elements are very similar to those of prokaryotic elements in certain respects. Both classes of elements are flanked by a duplication of the target site sequence of characteristic length and in many cases the termini of both classes of elements consists of either inverted or direct repeat sequences, which presumably serve as recognition sites for transposition functions. These structural similarities indicate that some aspects of the mechanism of transposition are the same in plants and prokaryotes.

Assuming that excision is an integral part of the transposition process in plants, then examination of DNA sequence that arise by excision of a transposable element should provide an insight on the mechanism of transposition. Two sources of such excision products are available. First, one can examine the relevant sequences of revertants that arise due to the excision of a transposable element in the germinal tissue of an appropriate mutant. Alternatively excision products in the form of cDNA or genomic clones can be isolated from the somatic tissue of unstable mutants in which the element is known to be freqently excised during development. Sequences from germinal revertants represent a selected subclass of excision products, namely those that have led to restoration of wildtype expression of the originally mutable locus. Sequences derived from the somatic tissue of unstable mutants, on the other hand, will include a wider variety of excision products.

A model for transposition and the sequence divergence generated by visitation of a locus by plant transposable elements is discussed with respect to the molecular evolution of new gene function.

14. Transposable elements controlling I-R hybrid dysgenesis in *Drosophila*

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Hybrid dysgenesis is the production of abnormal characteristics in the progeny produced when particular strains of *D. melanogaster* are crossed in an appropriate fashion. These traits include partial or complete sterility and increased frequencies of mutations and chromosome rearrangements. There are two independent systems of hybrid dysgenesis, P-M and I-R. P-M dysgenesis is produced by crossing M, maternal, strain females with P, paternal, strain males. In the I-R system R, reactive, strain females must be crossed with I, inducer, strain males. The progenies of all other crosses are apparently normal.

The properties of P and I strains are controlled by transposable elements known as P factors and I factors, respectively. We have cloned and characterised I factors at the molecular level. They are 5·4 kb long, and are present in 10–15 copies in the genomes of inducer strains. Incomplete I factors are present in all strains of *D. melanogaster*. We have determined the complete base sequence of one I factor, and have analysed the ends of several others. The structure of the I factor is unlike that of any other transposable element in *D. melanogaster* which suggests that it may have a novel mechanism of transposition.

16. Retroviral DNA integration and its relationship to transposition

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The retrovirus family is comprised of RNA-containing viruses which replicate through vertebrate host cells via DNA intermediates. A central feature of the viral replicative cycle is the insertion of viral DNA into the genome of the host cell. Moreover, the overall structure of this integrated DNA parallels that of the "retrotransposon" class of eukaryotic transposable elements such as the Ty elements of yeast and the *copia* family of *Drosophila*. In both cases coding sequences are flanked by long terminal repeats (LTRs) with the LTRs functioning in the *cis*-regulation of gene expression and nucleic acid replication. In addition, viral DNA insertion and retrotransposon transposition both result in the formation of short direct repeats in the cell DNA at the target site. Retrovirus integration requires two genetic loci located in the viral DNA. These are the *cis*-acting *att* site which becomes joined to the cell DNA and the *int* locus which encodes a protein (integrase) that mediates insertion through interaction with the *att* site. Regions hŏmologous to viral DNA encoding integrase, reverse transcriptase, protease, and a DNA binding protein have also been found in one or more of the retrotransposons. We have undertaken complementary genetic and biochemical approaches to study the integration process. A series of point mutants in the viral *att* site were constructed which profoundly diminish integration. In some instances reestablishment of integration can be effected by construction of compensatory second site mutations in *att*. We have also generated *att* sites from several retrovirus species and the putative *att* sites from two retrotransposons to determine whether viral integrase can recognise heterologous *att* sites *in vivo*. To begin a biochemical characterisation of the integration process, we have expressed and partially purified integrase in an *E. coli* expression vector. This enzyme appears to have DNA binding activity *in vitro*. With regard to expression of integrase and reverse tanscriptase, translation is complex in that amber codon suppression at the *gag-pol* junction is required for some retroviruses while frame shifting is required for expression in other viruses. We have devised a transient assay to study the translational amber codon suppression of the *gag-pol* junction in a variety of cell types.

17. The bovine leukemia provirus: its role in leukemogenesis

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Bovine leukemia virus (BLV), a retrovirus, is the etiological agent of a chronic lymphoproliferative disease of cattle called bovine leukosis. The tumor cell genome always contains at least a portion of a BLV provirus, a transposon-like structure devoid of cellular oncogenes. These proviral sequences appeared to be integrated at many different locations in the bovine genome (i.e. on different chromosomes). More surprisingly, they are not expressed even in established cell lines derived from tumor bearing animals and do not promote the expression of neighbouring cellular proto-oncogenes.

The recent discovery of a px region containing open reading frames at the 3′ side of the viral genome and the presence of tans-acting factors in BLV-producing cells allow us to postulate that leukemogenesis by GLV might be due to the trans-activation, through viral coded products, of key genes involved in cellular proliferation. However, the lack of viral expression in tumor cells most probably indicates that the trans-acting factors play their role during the initiation of the transformed state and are not required to assure its maintenance.

18. Genetics education at the school/university boundary

A discussion workshop organised by B. W. Bainbridge

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This workshop will continue discussions started in Edinburgh in March 1985. The aims are to improve the teaching of genetics at "A" and first year University levels, to influence the genetics contents of the syllabus and to survey the methods by which the subject is examined. Speakers are expected to include:

D. Devey (Cadbury College, Solihull) on "Genetics in GCSE and "A" level: how much should be included and what should be left out".

S. Baumberg (Leeds University) on "Survey of Genetics Examination questions".

A. Radford (Leeds University) on "In-service training in genetics for Teachers".

B. Bainbridge (King's College, London) on "Future trends in genetics teaching".

Short contributions from the floor will be welcome and participants are asked to contact B. W. Bainbridge before the workshop. Demonstrations of teaching resource materials will also be welcome. Copies of the report of the discussion forum at the meeting on 31 March 1985 in Edinburgh University will be available at the workshop.

19. Efficient detection of single copy DNA sequences on Southern blots with biotinylated probes

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Various human X-linked DNA variants (length 1·25–2·5 kb), labelled with a biotinylated nucleotide (uridine), were used as probes to detect single copy DNA sequences on Southern blots. The method involved has two main steps, the incorporation of the biotinylated nucleotide into DNA by nick-translation and the detection of this DNA by incubation with streptavidin and the biotinylated polymer of cal intestinal alkaline phosphatase. Compared with the method utilising radioactive probes, the biotin method has several advantages. These include a higher probe concentration allowing increased sensitivity without causing high background and shortening of both the hybridisation and detection time. Biotinylated probes are stable and can be produced in bulk. The method is reproducible and able to detect single copy DNA sequences in 2·5 μg of human genomic DNA on Southern blots using commercially available reagents.

20. Expression of an invertible region from *Staphyloccocus aureus* in *Escherichia coli*

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The invertible region (3.4 Kb, including 650 bp inverted repeats) of the staphylococcal plasmid pI9789 *bla*I, *bla*Z, *seg*-I has been cloned into the *E. coli* vectors pRW33 and pACYC184. Inversion occurs and is independent of the *E. coli pin* function.

A 3·9 Kb deletion (ΔD) adjacent to the invertible region of pI9789 *bla*I, *bla*Z, *seg*-I does not prevent inversion in *Staphylococcus aureus*, but clones derived from it are "frozen" in either orientation in *E. coli*. The pRW33 clones are, however, unstable and re-arrange due to interaction between one end of the invertible region and a specific vector sequence.

Diploids have been constructed in *E. coli* by transforming a pRW33/EcoC: pI9789 *bla*I, *bla*Z, *seg*I, ΔD "frozen" orientation clone with an "inverting" clone of EcoA:pI9789 *bla*I, *bla*Z, *seg*-I in pACYC184. In such diploids the invertible region of the ΔD clone now inverts and it is concluded that a trans-acting factor is produced by the non-deleted fragment.

Experiments to locate the gene(s) involved are in progress.

21. Cobalt resistance in *Aspergillus nidulans*

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The response of the ascomycete fungus *Aspergillus nidulans* to cobalt ions has been tested. Mutants

have been isolated which are resistant to the presence of cobalt as shown by their increased ability for hyphal growth. The evidence suggests that the resistance of most of these mutants is due to the production of pink crystals in the growth medium.

An X-ray microprobe analysis reveals that these crystals contain cobalt, calcium and sulphur. On solid media containing high levels of cobalt, mutant colonies have a characteristic "target" morphology with concentric rings of normal growth and hyphal growth without conidiation. This is most easily explained by the organism exuding a compound which diffuses ahead of the hyphae and precipitates cobalt to produce a metal-free zone. The amount of cobalt precipitated is greatest in the regions just outside the normal growth rings. Similar growth habits have been observed for natural copper-tolerant isolates of certain wood-decaying fungi (E. B. G. Jones, personal communication), which can exude liquid containing up to 0.1 M oxalic acid.

22. Hypervariability of Y-chromosomes as a result of structural rearrangement

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Hypervariability of sex-chromosomes, by comparison with autosomes, has been noted in groups as diverse as mammals and reptiles in systems with both male and female heterogamety. This contrast is particularly marked in the dioecious angiosperm *Rumex acetosa* with an XX/XY_1Y_2 sex-determining mechanism. The two Y-chromosomes are heterochromatic with the exception of a minute terminal pairing segment. In both, the centromere can occupy any position within the central 40 per cent of the chromosome but is excluded from the two distal 30 per cent regions. Centromere location is so labile that 68 U-variants have been identified in only 270 males. All populations are highly polymorphic with a minimum of four morphs in samples of 10 males. The Y-chromosomes are constant in size so variants must be generated by structural rearrangments of the central 40 per cent, not by quantitative changes in heterochromatin. In addition, the euchromatic pairing segment appears to switch ends with a high frequency. The origin of this massive variability in Y-chromosome organisation will be discussed.

23. Linkage: a computer assisted learning package for the BBC microcomputer

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This package of six computer programs has been developed for use by sixth-form and first-year undergraduate biologists. The package is intended to be a revision aid for linkage and chromosome mapping, and to supplement rather than replace a lecture course. As the lessons progress the emphasis is increasingly on problem solving rather than revision of elementary concepts.

The first two lessons are reviews of mitosis and meiosis with graphical simulations of chromosome behaviour. The third lesson compares patterns of inheritance for linked and unlinked genes, and considers the relationship between recombination frequency and map distance. The fourth lesson describes the estimation of recombination frequencies for two-point and three-point backcrosses, and the techniques used to produce chromosome maps are illustrated by worked examples and questions. Interference and coincidence are dealt with in the fifth lesson, and the final lesson contains two practice mapping problems.

A number of questions, both conceptual and numerical, have to be answered. Responses are validated and appropriate remedial tuition given depending on the type of incorrect answer. When calculations are required, the display is split so that the lower half functions as a calculator from which answers are submitted to the main program.

24. Human glyceraldehyde-3-phosphate dehydrogenase: expression during muscle development

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in glycolysis, which is

active in all human and other mammalian tissues. It is especially important for anaerobic energy production in skeletal muscle, in which high levels of activity are found and GAPDH can be identified as a major protein. In this study we have examined GAPDH in human muscle at various stages of development by parallel enzyme assay, isozyme analysis and assessment of mRNA levels. Quantitation by dot blot hybridisation of GAPDH mRNA revealed that levels in adult skeletal muscle are 12-fold greater than in fetal skeletal muscle at 10 weeks gestation. mRNA levels increased by only about 1·5-fold between 10 and 20 week gestation, indicating that the very high levels characteristic of adult limb muscle appear relatively late in development. In contrast, GAPDH mRNA levels in cardiac muscle were about equal in fetal and adult tissue. GAPDH enzyme activity levels correlated well with mRNA levels in all tissue examined. Isozyme analysis showed no evidence for fetus- or tissue-specific GAPDH forms.

25. The structure of a Chinese hamster glutamine synthetase gene

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The structure of an amplified glutamine synthetase (GS) gene from the Chinese hamster has been elucidated by sequence analysis of cloned genomic and cDNAs. The major mRNA comprises c.2810 bp encoding a presumptive protein of 372 amino-acids. This amino-acid sequence shows considerable homology with other eukaryotic and bacterial GS sequence, although homology with other ATP-hydrolysing enzymes is poor.

The gene is interrupted by 6 introns including one in the 5′ untranslated region. Two of the introns have *Alu* sequences at their 3′ ends. Two mRnAs of 2·8 kb and 1·4 kb are produced by the use of alternative polyadenylation sites.

26. Developmental expression of carbonic anhydrase III

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The carbonic anhydrases comprise a multigene family whose members exhibit different patterns of tissue specific expression. In man, there are three distinct and well-characterised monomeric zinc containing isozymes, CAI, CAII and CAIII. Previous studies on human CAIII expression using enzyme assay or immunological techniques suggested that this isozyme is restricted to skeletal muscle and as such may be a useful marker in muscle development and disease. We have investigated this tissue specific pattern of expression by Northern analysis of mRNA from various human tissues using a full length cDNA clone for human CAIII, pCAIII15. Analysis of mRNA from human muscle reveals a major component of 1·8 kb and several minor species of higher MW thought to represent partially processed precursors. Quantitative RNA analysis shows low levels of CAIII message in cardiac and smooth muscle and a developmental increase in skeletal muscle CAIII RNA from 3 per cent of adult levels at 10 weeks of gestation to 20 per cent of adult levels at around 20 weeks. These results are in good accord with protein analysis by immunoblotting. Expression of CAIII in myogenic cell lines has also been examined.

27. A transposon-like element conferring resistance to DNA damage in *E. coli*

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The *umuCD* gene of *E. coli* encodes mutagenic DNA repair. This process repairs DNA damage, so conferring resistance, but does so with an inherent possibility of mutating the repaired sequence. Southern hybridisation analysis of genomic DNA from modern hospital and 'pre-antibiotic era' *E. coli* shows that the *umuCD* gene is part of a transposon-like element. *umuCD* is at one end of a constant 12·5 kbp tract which is flanked by restriction site polymorphism. Amongst changes detected are flanking deletions. An ACGAAAA concensus sequence for Tn3-like transposon termini can be found at the end of the constant element. However the typical terminal GGGG is not found and inter-plasmid transposition could not be detected in a co-integrate assay. The element showed no cross-hydridisation with Tn, Tn1000, Tn21 and Tn2501. It is distinct from the nearby defective phage E14.

28. Structure, function and regulation of nodulation genes in *Rhizobium leguminosarum*

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In the *R. leguminosarum* symbiotic plasmid pRL1JI, nodulation and host-range genes had been shown to lie within a region less than 10 kb in size located between two clusters of *nif* genes. Transfer of this 10 kb region to other species of *Rhizobium* cured of their resident symbiotic plasmids or to *Agrobacterium tumefaciens* conferred the ability to nodulate peas and Vicia, the normal host legumes for *R. leguminosarum.*

This nodulation region of pRL1JI has been analysed, using genetic and physical techniques, by sequencing the DNA; the coding regions corresponding to eight genes (*nodABCIJDF* and *E*) have been identified and the effects of mutations in these genes were determined. A novel, wide host-range *lac* fusion plasmid was made and by constructing fusions in which individual nod genes were fused to *lacZ* the transcriptional organisation of these eight genes was established. In particular, it was shown that the transcriptional activation of *nodABCIJ* and *nodFE* required a factor present in the root exudate of peas plus the regulatory gene *nodD.*

29. Role of *rec* mutants of *Escherichia coli* K–12 in amplification of a chromosomal unit flanked by ISI elements in direct repeat

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A duplication of the ISI unit in which the *arg*F gene is located responds to selection for OTCase overproduction by amplification to approximately 45 copies. We are studying effects on formation of the amplification and on its stability, of genes known to be involved in homologous recombination in *E. coli* (*rec*A, *rec*BC, *rec*F, *sbc*B). Neither amplification or segregation occurs in a *rec*A or in a *rec*BC*sbc*B background.

30. Karyotypes of various laboratory and commercial yeasts by orthogonal-field alternating gel electrophoresis

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We have subjected 25 strains of *Saccharomyces* and 7 strains of other yeasts to analysis by OFAGE, which can separate and allow sizing of individual chromosomes (Carle and Olson, *P.N.A.S.* 82, 3756, 1985).

All but one of the *Saccharomyces* strains showed the general pattern of 12 or more bands in the range 200-1600 kb, with the majority representative of chromosomes 1000 kb. There were, however, extensive chromosome polymorphisms among many strains. Type strains of *S. cerevisiae, S. carlsbensis, S. uvarum* and *S. bayanus* and most commercial brewing, baking, wine and distilling strains showed different karyotypes. Many of the latter gave numbers and intensities of bands consistent with polyploidy or aneuploidy. Densitometer profiles of their gels provide a convenient characteristation of karyotypes of these industrial strains.

Yeast other than *Saccharomyces* and the strain types as *S. kluyveri* gave only a few bands, of sizes >1000 kb. The numbers of bands representative of larger chromosomes were: *C. albicans*, 4; *C. utilis*, 1; *H. wingei*, 2; *K. lactis*, 3; *P. canadensis*, 3; *Schw. occidentalis*, 4; and *S. kluyveri*, 3. The latter result suggests a genus other than *Saccharomyces.*

Bands of *Saccharomyces* strains are being assigned to known chromosomes by hybridisation with gene probes.

31. Biochemical analysis of a nonpathogenic mutant of *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* causes black rot of Brassicas. Prototrophic mutants have

been obtained which cannot infest *Brassica campextris* and several of these have been complemented by genomic clones of pathogenic *X. campestris* in the broad host range cosmid vector, pLAFR1 (Daniels *et al.*, *J. Gen. Microbiol.*, **140**, 2447, 1984; Daniels *et al.*, EMBO J., 3, 3323, 1984). Tn*5* mutagenesis of one of these clones (pIJ3000) indicates that about 10 kb of this plasmid is essential for infection of *B. campestris* (Turner *et al.*, *Molec. Gen. Genet.*, **199**, 338, 1985).

Results presented in this poster indicate that the "pathogenicity genes" of pIJ3000 are concerned with the export of enzymes (in particular polygalacturonases) from the bacterial cells. Further, comparison of membrane fractions from pathogenic and non-pathogenic Tn*5* insertions demonstrates major differences in their polypeptides.

32. Spontaneous transpositions of large chromosome segments in *Aspergillus nidulans*

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Duplications generated at mitosis can be selected readily in *A. nidulans* on the basis of gene dosage effects. Among the vigorous "revertant" sectors of certain leaky mutants grown on minimal medium, a proportion have duplication of a large segment bearing the leaky allele. In the first such series analysed, selected *via adE20*(IR), the majority had a large terminal segment of IR duplicated, and transposed in uninverted orientation, to the terminus of IIR (Sexton and Roper, *J. gen. Microbiol.* **130**, 583, 1984; Daud *et al.*, *Genetics* **110**, 229, 1985). In duplication strains selected from *ileA4*(IIR), the transposed, duplicate segments had random locations among the eight linkage groups when the parent was translocation free; but in strains selected from a parent with a particular translocation, T(VI, VII), there was a specific translocation-associated site of transposition (Marshall and Roper, *Heredity*, **53**, 554, 1984).

These transpositions show some parallels with, and some sharp differences from, transposition phenomena in prokaryotes and certain eukaryotes.

33. Regulatory interactions between segmentation genes during *Drosophila* embryogenesis

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The genes *fushi tarazu* (*ftz*), *hairy* (*h*) and *engrailed* (*en*) are all required during embryogenesis in order to establish the correct segmental organisation of the *Drosophila* embryo. Transcripts from these genes accumulate in periodic patterns at the blastoderm stage of development. We have found that generation of the normal pattern of *ftz* RNA requires the *h*+ function; in contrast the μ pattern forms normally in the absence of *ftz* activity. In addition expression of *en* is altered in both *h* and *ftz* mutant embryos. The nature of these changes and the cuticular phenotype of *h* mutant larvae suggest that both *h* and *ftz* phenotypes are a consequence of incorrect compartmentalisation and that the effect of *h* is mediated via *ftz* and other genes.

34. Molecular genetics of the *kruppel* gene in *Drosophila*

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35. The transfection and analysis of cloned genes in *Drosophila* cells

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Many of the later-expressed *Drosophila* genes are activated by the steroid hormone 20-hydroxyecdysone. Transfection and co-transfection are methods of choice for the molecular analysis of the gene organisation involved in this tissue and temporal regulation because hormone levels cannot be controlled in the developing organism. Since the hormone receptor protein may be species specific, transfection must be into *Drosophila* cells which are themselves hormone responsive. We have chosen to examine the organisation of a *Drosophila* yolk protein (*yp*) gene which is normally expressed in the fat body of adult females after a hormone pulse. We have developed a selectable vector from the *Drosophila* transposable element *copia* and the bacterial *gpt* gene, which allows selection of transfected cells in a HAT-type

medium. The *yp* gene is co-introduced into hormone responsive cells which do not make yolk. From 10-200 copies of the plasmid become integrated into the cell genome, predominantly as head-to-tail homopolymers. There is some constitutive expression of the *Yp* genes under these conditions, but about half the transfected clones respond to the hormone with a 5-10 fold increase of *yp* RNA transcription. There is thus no tissue restriction of *yp* gene expression under these conditions. The "upstream" sequences of the gene are necessary for this expression, and when they are combined with bacterial *gpt*, *CAT* or *-gal* structural genes, the hormone activates transcription of the latter. Further, the activation of these foreign genes can be analysed by their transient (non-integrated) expression in cells, permitting the rapid analysis of the promoter/activator sequences involved.

36. Analysis of genetic regulation using transgenic mice

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The orderly development of the mammalian embryo requires that appropriate sets of genes be activated in a variety of differentiating cells. Throughout development, cells must also maintain the ability to respond to signals which can modulate their pattern of expression. These issues are of particular interest in the case of genes that are expressed in more than one tissue, each of which regulates the gene in a different manner. An example of this type of gene is the alphafetoprotein (AFP) gene. It encodes a major serum protein activated early in mouse development in the visceral endoderm of the yolk sac, the foetal liver and the foetal gastrointestinal tract. The AFP is developmentally regulated in that its level undergoes a 10,000 fold decline shortly after birth. This decline is under genetic control by at least one trans-acting regulatory locus, termed *raf*, which determines the adult basal level of AFP.

To approach the molecule mechanisms underlying the regulation of the AFPgene, the gene has been introduced into the mouse germline via microinjection. Transgenic mice provide the ideal experimental system for addressing questions of gene activation, because they allow a detailed analysis of regulated gene expression in all tissues throughout development. Using this approach, it has been found that the introduced AFP genes are activated in only the appropriate tisues at the proper time in development, and that they also are developmentally regulated in the liver after birth. Furthermore, deletion analysis of constructs has shown that multiple sequences spread over a 7 kb region 5′ of the AFP gene are involved in the tissue specific activation of the gene. These elements or sequences have properties similar to enhancer elements. The roles of these in AFP gene regulation will be discussed.

37. Determination of vulval cell lineages in *Caenorhabditis elegans*

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In *C. elegans* vulval development involves six precursor cells, P3.p-P8.p, each of which has the potential to express any one of three cell lineages, designated 1°, 2° and 3°. The proximity of a regulatory cell, the anchor cell, governs which lineage is expressed. Homeotic genes have been identified that affect the determination of Pn.p cell lineages. Mutations in genes such as *lin-2* and *lin-10* result in the expression of 3° lineages by cells which would otherwise undergo 1° or 2° lineages. These genes are likely to be involved in either the reception of the anchor cell signal or in the induction of 1° and 2° cell lineages. Mutations in genes such as *lin-15* result in the transformation of 3° to non-3° cell lineages. Finally, mutations in *lin-31* result in the uncontrolled expression of 1°, 2° and 3° lineages such that the same Pn.p cell in different animals can express any of the three lineages.

We are initiating a molecular analysis of the determination of vulval cell lineages by using transposon-mediated mutagenesis to clone the genes described above. There are mutator strains of *C. elegans* which have an enormously increased frequency of transposition of the major transposon, Tc1. From one of these strains, we have isolated 26 putative transposon-induced mutations in five genes that affect the determination of vulval precursor cell fates; *lin-2, lin-10, lin-12, lin-15* and *lin-31*. Analysis of the mutations in the genes *lin-12* and *lin-10* has been the most extensive. Using a

probe for the *lin-12* gene (previously isolated by Iva Greenwald), Southern blotting analysis suggests that the spontaneous *lin-12* mutation is caused by the inxertion of Tc1 copy into the gene. For the spontaneous *lin-10* allele, a Tc1 copy is located inseparably close to the *lin-10* mutation on the genetic map, suggesting that this Tc1 caused the mutation by insertional mutagenesis. Using Tc1 as a probe, *lin-10* has been cloned. Similar analyses for the spontaneous *lin-2, lin-15* and *lin-31* mutations are in progress. Thus, by isolating transposon-induced alleles, it should be possible to clone genes that have been identified solely at the genetic level.

38. Expression of a mouse homoeo-box gene during embryonic development

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The homoeo-box is a short segment of DNA encoding a putative DNA-binding protein domain. Homologues of the homoeo-box, encoding highly conserved domains, are present in a number of genes of *Drosophila* known to be important in regulating segment number and segment identity. Homoeo-boxes are also present in vertebrate genomes and are similarly well-conserved. With the anticipation that this conservation reflects a role in regulating development for these genes, I and others have isolated some of these genes from libraries of mouse DNA. I have shown that one gene, H24.1 is expressed in a spatially and temporally regulated manner during embryogenesis. It is expressed in the neural tube of 12.5 day embryos and in adult kidneys. The timing of the switching on and off of the gene in different tissues is under investigation.

Methods are being developed which will allow specific insertional mutagenesis of H24.1 (or any other gene of choice) in embryonal stem cells *in vitro*. The stem cells so modified can then be reintroduced into embryos, which will give rise to chimaeric mice. Breeding from these mice will give rise to offspring heterozygous for the mutated gene, from which homozygotes can be made, to test the effect of an absence of H24.1 expression on development. This will be a powerful tool for investigating the function of cloned DNA *in vivo*.

40. The molecular mapping of the mouse X chromosome

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Microdissection and microcloning of a proximal region of the mouse X chromosome has yielded some 550 genomic clones (Fisher, Cavanna and Brown, *Proc. Natl. Acad. Sci. USA* **82**, 5846, 1985). This bank of X-specific microclones is being used for the detailed molecular mapping of the proximal region of the mouse X chromosome. A parental cross of a wild *Mus spretus* mouse with a *Mus domesticus* mouse carrying the X-linked coat colour mutants Tabby (*Ta*) and Harlequin (*Hq*) yielded four female progeny each of which was backcrossed to inbred *mus domesticus* mice. 233 progeny were produced in these backcrosses segregating either for *Ta* or *Hq*. The wild *Mus spretus* species is highly diverged from *Mus domesticus* and microclones show abundant restriction fragment length variants between the *spretus* and *domesticus* genomes. The 233 backcross progeny were scored for the *Ta* and *Hq* genetic markers and for *spretus* or *domesticus* microclone restriction fragment length variants. The segregation of markers and microclone variants in the backcross progeny allows an accurate positioning of the microclones on the X-chromosome genetic map. It is hoped that the provision of such detailed molecular maps will aid in the isolation of X-linked genes of known phenotype but unknown gene product.

41. Analysis of the conjugation system of *Staphylococcus aureus*

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Some plasmids of *Staphylococcus aureus* that confer resistance to gentamicin also carry information for their own conjugal transfer. This system may have great significance in the spread of staphylococcal infections. This report describes the application of two approaches used to locate such regions of conjugative plasmids.

The first approach involves the cloning of restriction enzyme fragments into suitable *S. aureus* vectors. Such analysis allowed the location of the regions of the plasmid (8325-2) conferring resistance to gentamicin and to ethidium bromide, but no clone acquired the ability to conjugate.

The technique of transposon mutagenesis produced a series of mutants with altered conjugation frequencies. These results lead to the tentative conclusion that at least two well-separated regions of the plasmid are involved in conjugation.

42. Palindrome inviability in bacteriophage lambda

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Palindromic DNA sequences of more than a few hundred base pairs in length confer inviability to their carrier replicons. A 530 bp palindrome in bacteriophage lambda confers inviability in *RecBC*$^+$ *SbcB*$^+$ but not *recBC sbcB E. coli* hosts. The latter strains can therefore be used to prepare high titre lysates of the phage. Using this system, we have shown that DNA activity is required for loss of palindromic DNA. Furthermore, we have measured the extent of DNA replication, using a lysate modified against cleavage with endonuclease *Eco*RI and have shown that the DNA carrying the palindrome is replicated slowly. Conversely, these results demonstrate that inviability is not caused by cleavage of cruciform DNA.

43. F factor-mediated activation of ISI elements in the chromosome of *Escherichia coli* K-12

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Analyses of the *arg*F region of *E. coli* K-12 have shown that it is flanked by ISI elements in direct repeat and that the unit can respond to selection for overproduction of the enzyme ornithine transcarbamylase by amplification. A novel feature is that the occurrence of the amplification depends on the F factor in *cis*; less surprisingly, it also depends on the *rec*A product. We have shown that the F factor influences the initial stage only, when the ISI elements recombine to give a duplication. This duplication appears to be unusually stable (Jessop, A. P. and Clugston, C., *Molec. Gen. Genetics* **201**, 347, 1985).

45.

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46. The mini-circle sequence, a transposable element of *Streptomyces coelicolor* A3(2)

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The mini-circle sequence of *Streptomyces coelicolor* A3(1) exists as two linear copies integrated into the host chromosome and also as a free 2.6 kb CCC species with an apparent copy number of one per 20 chromosomes.

When the entire mini-circle sequence was inserted into KC514 (a derivative of the temperate phage C31 unable to lysogenise host strains because the phage attachment site has been deleted) the resulting phase lysogenised not only *S. coelicolor* but also most other C31 hosts. Lysogens of eight *Streptomyces* species (which naturally contained no homology to the mini-circle) possessed linear, integrated prophages with termini apparently identical to those of the linear mini-circle copies of *S. coelicolor*, suggesting that the mini-circle sequences inserted into the recombinant phage actively directed its integration into host genomes.

When a 1·8 kb fragment of the mini-circle was inserted into KC515 the resulting phase integrated into the *S. coelicolor* genome by homologous recombination with resident mini-circle sequences. A selectable marker on the phage allowed a loci of the chromosomal mini-circle copies, containing inserted prophages, to be genetically mapped in *S. coelicolor* lysogens.

Recently, a recombinant molecule consisting of the *Escherichia coli* cloning vector pBR327, the entire mini-circle and a thiostrepton resistance determinant transformed *S. lividans* to thiostrepton resistance. This system offers attractive possibilities for the stable cloning of foreign genes into streptomycetes at single copy number.

47. Target sequence requirements for high frequency transposition of TN7

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We have previously shown that Tn7 (a 14 Kb bacterial transposon encoding trimethoprim, streptomycin and spectinomycin resistance) has a unique preferred site of insertion in the *E. coli* chromosome with a transposition frequency of 95–100 per cent (Lichenstein and Brenner, *M.G.G.* **183**, 380, 1981; Lichenstein and Brenner, *Nature*, **297**, 601, 1982). This site has been localised to the transcriptional terminator of the glucosamine synthetase gene (Walker *et al.*, *J. Biochem.*, **224**, 799, 1984). Other workers have reported preferred sites for Tn7 transposition in other bacteria (Fennewald and Shapiro, *J. Bacteriol.* **136**, 264, 1979; Thomson *et al.*, *J. Bacteriol.* **148**, 374, 1981; Ely, *J. Bacteriol.* **151**, 1056, 1982; Turner *et al.*, *M.G.G.*, **195**, 101, 1984; Bollcher *et al.*, *J. Gen. Microb.*, **131**, 2449, 1985).

In this study we show that Tn7 transposes to unique sites in the chromosomes of *Agrobacterium tumefaciens*, *Klebsiella pneumoniae* and *Serratia marcescens* with similar frequencies to *E. coli*. We have determined the DNA sequence of the insertion sites and compared them to our previously characterised *E. coli* site. In all cases Tn7 has transposed into the same site in the *glmS* gene. There is a strongly conserved sequence (GCG/AGGCAATT/G) at the point of insertion. To determine the precise DNA sequence required for efficient site-specific transposition we have constructed a series of deletions in the *E. coli* site. The vectors used (VHV12 and VHV22) consist of M13mp8 and a 1 Kb fragment of chromosomal DNA containing the attachment site in both orientations. This phage vector can be used as a target for Tn7 transposition by scoring for transduction of trimethoprim resistance (giving 10^{-3} resistant colonies per plaque forming unit contrasting with a frequency of 10^{-10} for control phage). Using this assay we have defined a 55 bp region of target DNA into which TN7 transposes with wild-type frequencies. We are currently characterising this sequence using site directed mutagenesis to determine which bases are required for site-specific transposition.