treatment (6 h) with CHM at the same concentration also presented 2 peaks but after 36 h and 44 h respectively, i.e. with the same interval between the 2 peaks. The same arguments as those used for the 1st peak are valid for the 2nd. They demonstrate that this 2nd limited population is composed of cells which were in the S and G_2 phase during the pulse labeling and CHM treatment, and entered mitosis later than the first population. On the other hand, the 2nd peak of the control curve (fig. a) represents cells which divide for the 2nd time after the pulse labeling.

Our results show that a critical examination must be carried out in order to explain the curve of the index of labeled metaphases. It could be assumed from a superficial comparison between the curves concerning untreated and CHM-treated cells that these 2 graphs have the same significance even if they are shifted in time, whereas in fact they represent different data. In the untreated roots, the meristems were composed of cell populations progressing through the cell cycle; the cells progressed at different rates, some cells being naturally arrested. In the CHM-treated roots, the cells were all stopped for different lengths of time according to the stage in which they were arrested. It is known[5] that, in the case of the control (fig. a), the ^{3}H-TdR pulse-labeled cells enter mitosis for the first time during the 1st peak and for the 2nd time, after passing through a full cell cycle, during the 2nd peak. But, as we have shown, with a CHM treatment, the 1st and 2nd peaks (fig. b) belong to the 1st cell cycle and are composed of S and G_2 cells. Our data also show that CHM, as opposed to other protein synthesis inhibitors, has a special action on the cells which were in S and G_2 phases at the time of treatment. A comparative study of CHM, puromycin and aurin tricarboxylic acid[7] indicates that the 3 inhibitors induce a reversible blockage in G_1 and in G_2; but whereas the last 2 inhibitors, when removed, let the G_2 cells divide first, a CHM induced blockage of G_2 cells lasts longer than that of S cells. CHM is known to affect cellular metabolism by inhibiting protein synthesis and by disrupting energy transfer[9,10], this may account for the different reaction of G_2 cells.

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A Y-translocation method for localizing enzyme genes on *Drosophila* polytene chromosomes

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Summary. X-Ray induced translocations between autosomes and the Y-chromosome giving balanced and aneuploid (partially trisomic) male offspring proved useful for a rather precise localization of enzyme loci in the subsections of the polytene chromosomes of *Drosophila subobscura.*

Electrophoretic techniques which allow the detection and separation of allozymes are now widely used in many fields of biology and medicine. The direct colinearity between the amino acid sequence of proteins and the DNA base sequence makes enzyme loci very useful tools for genetic analyses. Especially genetic variants, phenotypically visible as allozyme patterns after electrophoretic separation, are easily found in almost all organisms including man[2].

Working on the relation between allozyme and chromosomal inversion polymorphism in *Drosophila subobscura*[3], we became interested in determining the exact cytological location of the enzyme genes on the giant chromosomes. Various methods have been elaborated in order to localize enzyme loci on chromosomes, according to the cytological possibilities and the properties of the organism. In man and in mammals cell hybridization and subsequent cytological and enzyme studies have proved successful[4]. In *Drosophila melanogaster* many sophisticated marker strains and deletion-carrying chromosomes facilitate the cytogenetic localization of structural genes[5]. In situ hybridization of labeled DNA at the polytene chromosomes offers another possibility but requires prior cloning of the specific DNA[6]. For our special purposes, studying a species where neither enough marker strains nor a genetic library are available, we developed a Y-translocation technique which can be applied in principle to all animal species with polytene chromosomes, as far as laboratory breeding and crossing is possible.

Material and methods. The translocations were induced by irradiating male flies with an X-ray dose of 9000 rad (18,000 r/min). Separation of allozymes was done on horizontal starch gels according to Ayala et al.[7]. Chromosome analysis was performed on acetic-orcein stained giant chromosomes of larval salivary glands. The genetic variants of the enzyme genes to be localized were derived from samples of wild flies collected for our population genetic studies. Homozygous strains for different allozyme variants were established by inbreeding. As we had an adequate sample of wild flies from natural populations, variant alleles for all, even for 'monomorphic' loci, could be obtained easily.

Screening procedure. The basic idea of the technique is to produce Y-autosome translocations, to determine the breakage points on the giant chromosomes cytologically,

and to decide by genetic and electrophoretic analysis which enzyme genes have been translocated. Having examined a number of different translocations, the position of a gene on a specific chromosome can be more and more defined by adjacent breakage points until a rather small region or a group of bands remains as the possible location of the gene. The crossing procedure for the screening is shown in figure 1. A recessive visible gene marker (in the present example *cn*) is needed for each chromosome to be analyzed. Wild-type (+/+) males are irradiated and crossed to females homozygous for the recessive marker gene (*cn*/*cn*). The F_1-males are individually backcrossed to *cn*/*cn*-females.

As a result of the irradiation 3 possibilities have to be considered: a) no translocation, b) translocation of a part of the autosome without the *cn* locus; and c) a translocation including the *cn* locus. Without a translocation, wild-type and *cn* phenotypes appear in a 1:1 ratio in males and females of the F_2. In case the wild-type (+) allele of the *cn* locus has been translocated, only wild-type males and *cn* females are found in the F_2. If the translocated piece of the chromosome does not contain the *cn* locus, the F_2 males are either wild-type or *cn* but all females are *cn*. Hence translocations between the Y-chromosome and a marked autosome can be recognized easily by a quick inspection of the females of the F_2 offspring. If wild-type females are found, the culture is discarded. If only *cn* females are present, the breeding line is examined further. In our experiments 2–8% of the F_2 offspring proved to carry a translocation.

Localization of enzyme genes. For those enzyme genes which are present as different alleles on the irradiated chromosome and on the chromosome carrying the visible marker gene no further test-cross procedure is needed for deciding whether the gene locus was translocated or not. Individuals of the F_2-generation can be directly used for cytological and electrophoretic examination. Yet, translocation strains can be kept for further test-crosses. Thus, a sufficient number of translocation strains can be collected and used later when appropriate marker strains for other enzyme loci are available. The test-cross can be done simultaneously for several enzymes. Figure 2 describes a situation where 2 enzyme genes (*α*Gpdh and Mdh) are examined and 2 alleles are available for each of the 2 genes. In this case males carrying the translocation are crossed and backcrossed to females of a strain homozygous for different alleles at the 2 loci mentioned. In the offspring 2 types of males are expected: 1. The "balanced type", carrying a Y-chromosome with the autosomal piece together with the deleted autosome and the intact maternal autosome, and 2. the "aneuploid type", which possesses 2 intact maternal autosomes and in addition the translocated autosomal piece on the Y-chromosome.

In the example shown in figure 2 one of the genes (*α*Gpdh)

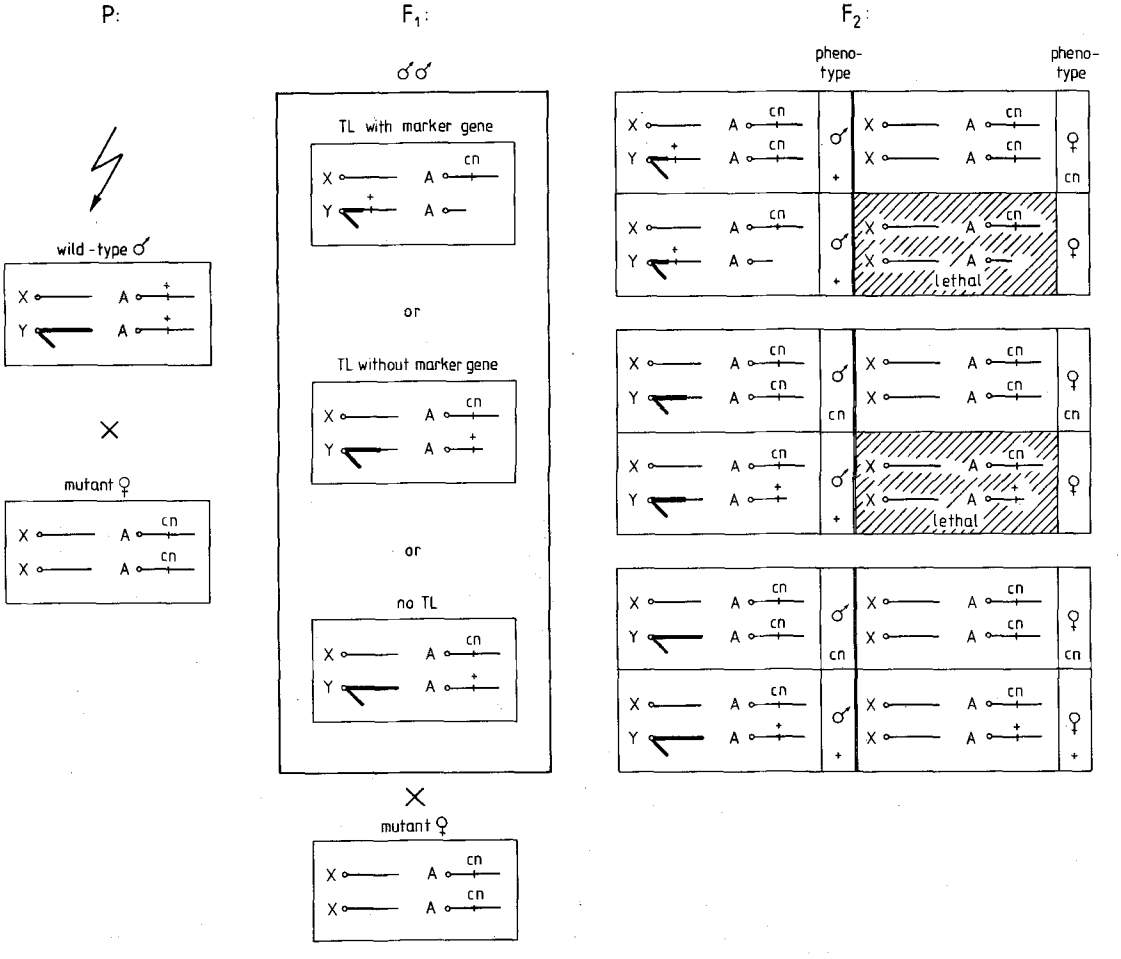

Figure 1. Screening procedure for Y-autosome-translocations. Irradiated wild-type males (+/+) are crossed to females homozygous for a recessive marker gene (e.g. *cn* = *cinnabar*). F_1 males are individually backcrossed to the marker strain. Since F_2 females receiving a deleted autosome from the father are lethal, Y-autosome translocations can be detected by examining the phenotypes of the F_2. (X, Y = sex-chromosomes, A = autosome, TL = translocation).

has been translocated to the Y-chromosome whereas the other one (Mdh) remained on the deleted autosome. As a consequence, the balanced males are heterozygous for both the translocated and the non-translocated gene. The aneuploids, however, are homozygous for the non-translocated locus but have the translocated gene in 3 copies of which 2 are coding for the slow (S) allozyme and 1 for the fast (F) variant. The electrophoretic pattern obtained for the dimeric enzyme αGpdh is in accordance with this assumption. The slow band which corresponds to the S+S homodimer and the hybrid band of the S+F heterodimer are more intensively stained than the fast band of the F+F homodimer (expected ratio: 4:4:1). On the other hand, the balanced males show the normal allozyme pattern of a dimeric enzyme in a heterozygote genotype.

An even more convincing proof for aneuploidy of an enzyme gene is demonstrated in figure 3. Three different variants were available for each of the 2 loci. In the case of Pgm, which is a monomeric enzyme molecule, the translocation was induced in a male homozygous for the S-allele which happened to be joined with the Y-chromosome. Males carrying this translocation were crossed to females homozygous for the allele F, and the F_1 males crossed to females homozygous for the allele M. As a result the balanced males of the F_2 are heterozygous S/M, but the aneuploid males possess 3 different alleles of the gene. As can be seen from figure 3a, all 3 genes are expressed. Similarly, aneuploid males with 3 alleles (S/M/F) can be obtained for a gene coding for a dimeric enzyme (αGpdh in fig. 3b). The intensity of the bands found in the electropherogram of the "tri-allelic" genotypes corresponds quite well to the expected 1:2:3:2:1 ratio of random combinations between the molecular subunits. (The medium band consists of the overlapping bands of the homodimer M+M and the heterodimer S+F). Yet, for our purpose it is more important that the presence of 3 or 5 different allozymes in the zymogram proves unambiguously that the enzyme locus was translocated.

The final step is, of course, the cytological examination. Examples are given in figure 4. Aneuploids are quite readily recognized by their giant chromosomes. The distal

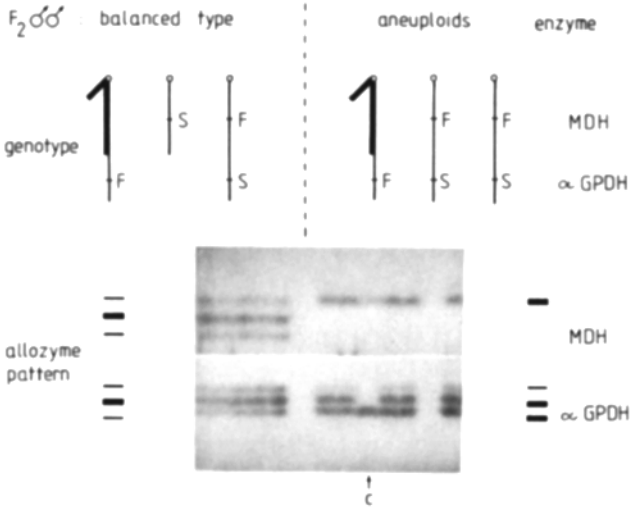

Figure 2. Offspring of a test-cross for the localization of enzyme genes. The irradiated autosome carried the alleles Mdh^S and $\alpha Gpdh^F$. The allozyme patterns found in the F_2 males indicate that the αGpdh-locus was translocated to the Y-chromosome whereas the Mdh-locus remained on the deleted autosome. (C, control fly from a standard strain of *D. subobscura*).

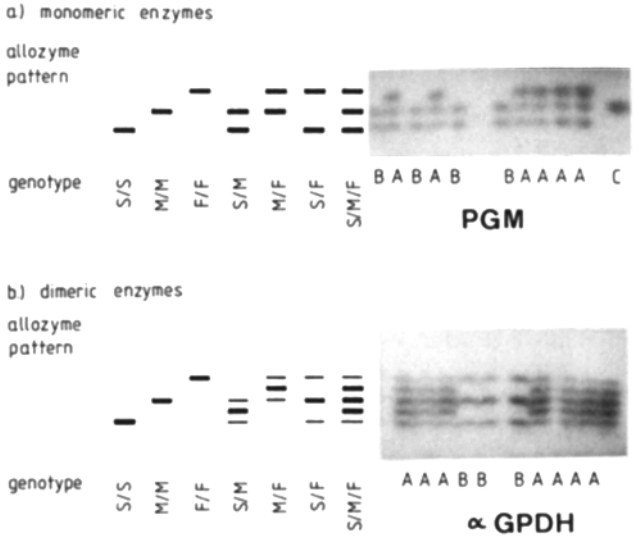

Figure 3. Allozyme pattern in aneuploid males with 3 different alleles (S/M/F). The possible allozyme patterns in homozygotes and heterozygotes are drawn schematically. The zymograms of balanced (B) and aneuploid (A) genotypes are shown in the photograph. (C, control fly from a standard strain of *D. subobscura*).

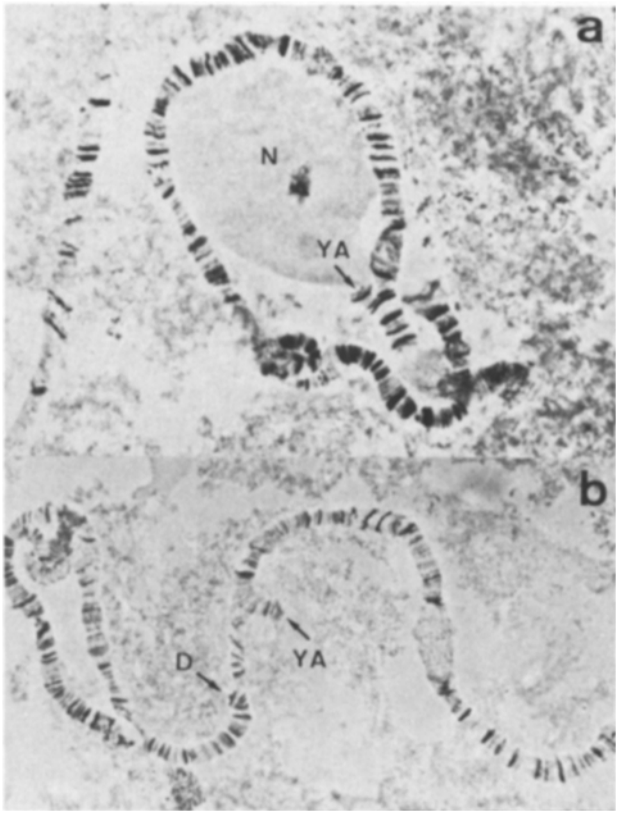

Figure 4. Polytene giant chromosomes with translocation breakpoints (indicated by arrows). *a* Aneuploid, distal end 3-stranded. *b* Balanced, one of the 2 strands interrupted. Both photographs show chromosomes J of *D. subobscura* from different translocation strains. N, nucleolus; YA, attachment site to the Y-chromosome which is not visible in polytene chromosome sets; D, distal end of the deleted autosome.

part of the chromosome in figure 4a is 3-stranded and one of the strands corresponds to the region translocated to the Y-chromosome. Since the Y-chromosome is not polytenic in the salivary glands but heterochromatic and incorporated into the centromeric heterochromatin, this strand is frequently associated with heterochromatin or with the nucleolus (as in fig. 4a). Balanced males have polytene chromosomes in which 1 of the 2 strands is interrupted at the position of the translocation break point (indicated by arrows in fig. 4b). Knowing which part of the chromosome is translocated and having the information from the allozyme pattern, a decision can be made on which part of the chromosome the gene must be situated. With enough different translocations a rather precise localization can be achieved.

To make things clear, the existence of autosome-Y translocations was deduced only from the way of inheritance of the marker genes and the enzyme loci. Further evidence comes from the salivary gland chromosomes of male larvae (e.g. partial triploidy in fig. 4) and the allozyme pattern of male larvae. Yet, no direct cytological proof from mitotic metaphase chromosomes exists. Further studies in this direction are planned.

Discussion. In our experience the method works quite well. Exceptional or unexpected results did, however, appear in a few cases. Autosome-autosome-Y translocations or insertion of an intermediate section of an autosome into the Y-chromosome or X-ray-induced inversions proved to be responsible for them. However, the normal, unambiguous situation described above is frequent enough to allow the exclusion of the exceptions from the experiments. Up to now we have examined about 50 different translocations involving all 4 autosomes of *D. subobscura*. We have never observed the repression of any of the different enzyme genes (Adh, Aph, Est, α-Gpdh, Hk, Idh, Mdh, Me, Pgm) due to the altered position in the heterochromatic Y-chromosome. Thus we are rather confident that this translocation technique can be applied not only to species of the genus *Drosophila* (we have unpublished data for *D. melanogaster*, *D. pseudoobscura*, *D. ambigua*, and *D. paulistorum*) but also to other genera of Diptera with a similar organisation of sex-chromosomes.

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Intramitochondrial inclusions in maturing and senescent muscle cells of rat myocardium[1]

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Summary. Intramitochondrial myelin-like structures were found in ventricular myocardial cells of the rat heart. These inclusions were found in both the first 2 postnatal weeks and in senescent stages. The origin of such myelin-like structures could be related to an age-dependent metabolic change, specifically to an inability to oxidize long-chain fatty acids described in both periods.

The major constituents of the cardiac muscle cell have been the subject of numerous electron microscopic investigations. However, less conspicuous components have received little attention. Recent investigations showed the appearance of cytoplasmic myelin structures during the first 2 postnatal weeks and in the senescent periods of the rat's life[2]. Also, intramitochondrial inclusions have been found in different tissues, and these have been related to the aging process[3] to pathological conditions[4], or to metabolic activity[5]. We describe here an intramitochondrial inclusion that could be age-dependent and related to metabolic changes.

36 male and female Wistar rats aged 4–15 days and 1, 3, 12 and 26–30 months, were used. Anesthesia was induced with ether and the hearts were perfused with Karnovsky's mixture[6], buffered with 0.2 M collidine. The material was postfixed in OsO_4, dehydrated and embedded in Epon 812. Ultrathin sections for electron microscopy were stained with uranyl acetate-methanol and lead citrate and studied in a Philips EM-301 electron microscope.

Myelin-like structures were found in the mitochondrial during the first 2 postnatal weeks (young animals). After that stage they were not seen in adult animals, but appeared once again in 2-year-old animals (senescent period). These myelin-like structures consist of highly osmiophilic material arranged in lamellae. The lamellae are composed of multilayers of osmiophilic bands regularly spaced at a distance of 40–50 Å (figs a and c).

These figures are similar in both young and senescent animals, and are also similar in form and in time of appearance to those previously reported in the cytoplasm[2]. When they are present within mitochondria there is a continuity between the myelin structures and the 2 mitochondrial membranes (fig. b). At high magnification the myelin-like structures seem to be formed from the inner membrane of the mitochondria (fig. c).

Mitochondrial structure undergoes drastic changes in relation to changing physiological activity; examples are the changes during the hibernation cycle of *Cylellus lateralis*[7], or those in cardiac muscle during experimentally induces hyperthyroidism[5]. In addition, different kinds of intramitochondrial bodies or inclusions have been reported in many different metabolic conditions; for example, intramitochondrial bodies have been found in bovine adrenocortical cells[8]. The number of inclusion bodies in the zona glomerulosa was increased by an uncompensated loss of body sodium[9] and by sodium restriction[10], and similar bodies appeared more frequently in the corpus luteum during early gestation[11]. These findings may indicate a relationship