Onset of mitosis after cell cycle blockage induced by cycloheximide in root tip cells[1]

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Summary. The cells in S-phase at the time of a short treatment with cycloheximide, inducing reversible arrest of the cell cycle, enter mitosis before those in G_2 and are divided into 2 subpopulations when cell proliferation resumes.

Previous experiments have shown that the protein synthesis inhibitor cycloheximide (CHM) prevents cell progress in garlic root meristems (*Allium sativum* L.) throughout the cycle: i.e., in G_1, S, G_2 and M. A short treatment with this drug induced temporary arrest of the asynchronous proliferating cells[3,4]. The reversibility of the cell proliferation blockage led us to study cell kinetics using the Quastler and Sherman[5] method of pulse-labeled metaphases following inhibitor removal. Previous data obtained after a 6-h treatment with the same inhibitor did not allow us to give a satisfactory interpretation of the curve showing the percentage of labeled metaphases[6]. The mean duration of the cell cycle and its parameters are established from 2 successive cycles; in the case of an untreated population, these are constant while in the case of a CHM-treated population, these 2 cycles have a very unequal duration. Previous[6] and present results as well as those obtained by other methods[7] were compared. This comparison led to a better understanding of the curve of pulse-labeled metaphases obtained after a CHM treatment. It showed the special behavior of S and G_2 cells treated with CHM during a short period.

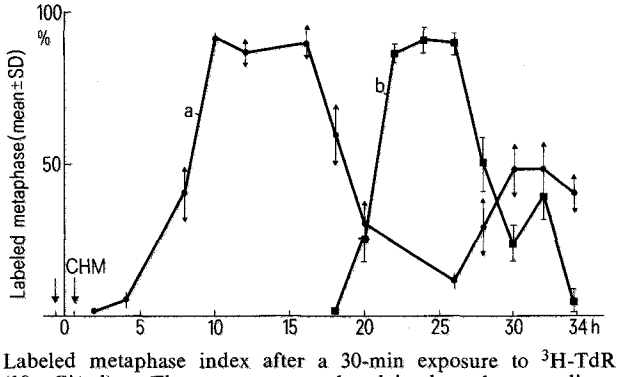
Material and methods. Garlic bulbs (*Allium sativum* L.) were grown in the dark at a temperature of 22 ± 1 °C in Knop medium diluted 2-fold. When roots were 2.5–3.5 cm long they were immersed in a tritiated thymidine (^{3}H-TdR) solution (C.E.A. Saclay France, at a concentration of 10 µCi/ml; sp. act. 25 Ci/mM) during 30 min. They were then washed in cold thymidine a 100 times more concentrated than the tritiated solution. A control sample of roots was replaced in the culture medium. Another set of roots was first treated with a CHM solution at a concentration of 10 µg/ml during 30 min and then replaced in the culture medium. At the end of the exposure period, 4 root tips (2 mm) were taken from each set every 2 h until the 34-h point. They were fixed and stained in acetic orcein then squashed under coverslips, and quick-frozen[8]. The slides were dipped into Ilford K_2 diluted 1:1 with distilled water. After a 15–20-day exposure in the dark at 4 °C they were developed (Kodak D 19). The percentage of labeled metaphases was determined by scoring 100 metapheses from 4 roots for each sample.

Results and discussion. The control curve of the percentage of labeled metaphases (fig. a) was similar to the one obtained in a previous experiment[6] (the mean values of the cell cycle parameters were as follows: T 23 h, S 11 h, G_2 6 h, G_1 3 h, M 3 h). It should be noted that the mitotic index of an untreated population, in a steady state, remains constant (10%) during the whole experiment, whereas after a short CHM treatment, no mitosis was observed from 8 h to 18 h after the end of the treatment; this long period was followed after 20 h by a progressive onset of mitosis. The curve of the percentage of labeled metaphases for CHM-treated roots (fig. b) showed several periods: a 1st peak with an ascending slope (18–22 h) with a maximum of 90%, stable between 22 h and 26 h, and a descending slope from 26 h to 30 h with a minimum of 24%, then a 2nd peak (30–34 h) with a maximum at 32 h about 40%.

1. Analysis of the 1st peak. The ascending slope of the 1st peak of the curve obtained after the CHM treatment (fig. b) started after 20 h. At this time, there were labeled and unlabeled metaphases but few cells were in mitosis (mitotic index = 4.5%). After 22 h, the maximum percentage, 90%, of labeled metaphases was reached with a mitotic index of 9%, demonstrating the almost exclusive entry into mitosis of cells which were in S, when treated. Previous data (fig. 5 in Lévy et al.[7]) support these results. They showed that under identical experimental conditions, but when roots were simultaneously treated with CHM for 30 min and labeled continuously with ^{3}H-TdR, the first metaphases observed, after 22 h, were all labeled. In the present experiment, the unlabeled metaphases observed simultaneously with the labeled ones, after 20 h, could be due to the conditions of exposure to the radioactive precursor (pulse-instead of continuous labeling) and/or to the time of the CHM treatment in relation to that of the labeling with ^{3}H-TdR (preliminary labeling instead of simultaneous labeling). In the case of pulse labeling prior to the CHM treatment, some cells, at the end of S, may be unlabeled and thus indistinguishable from G_2 cells.

The descending slope of the 1st peak of the control curve (fig. a) corresponds to the entry into metaphases of the cells which were in S and in G_1 at the time of the pulse labeling[5]. This part of the curve does not have the same significance for the treated roots (fig. b). Previous results obtained with other methods have shown that the unlabeled metaphases appearing between 26 h and 30 h were only G_2 cells. First, the former results, mentioned above[7] provided direct proof of this fact since, during a continuous labeling with ^{3}H-TdR, the unlabeled metaphases which appeared at this time could only be G_2 cells (G_1 and S cells being necessarily labeled when entering mitosis). Secondly, earlier results (fig. 9 in Lévy et al.[7]) showing that cells arrested in G_1 only enter mitosis 48 h after the end of the CHM treatment, corroborate this conclusion. Thus, when mitotic activity resumed, after cell cycle blockage with CHM, S cells were the first to enter mitosis followed by G_2 cells.

2. Analysis of the 2nd peak. The curve obtained after the CHM treatment (fig. b) revealed a 2nd peak with a maximum of 40% after 32 h, i.e. soon after the first one. In a previous experiment (fig. C in Benbadis et al[6]), the curve of the index of labeled metaphases established after a longer



Labeled metaphase index after a 30-min exposure to ^{3}H-TdR (10 µCi/ml). *a* The roots were replaced in the culture medium. *b* The roots were treated during 30 min with CHM (10 µg/ml) and subsequently replaced in the culture medium.

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,