The Effect of Chloral Hydrate upon Mitosis in *Aspergillus nidulans*

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INTRODUCTION

Although chloral hydrate has been used as an anesthetic since 1872, its effect on cell structures and its mechanism of action are not clearly known. Early investigators (Nemec, 1904; van Regemorter, 1916; Strasburger, 1907; Sakamura, 1916) have shown that chloral hydrate produces uncoordinated chromosome movements and c-mitosis i.e. colchicine mitosis (Levan, 1938). Ris (1949) reported that chloral hydrate causes the disappearance of the mitotic spindle in grasshopper spermatocytes. Using eggs of *Pleurodeles waltlii*, Sentein (1974) demonstrated that chloral hydrate alters the ultrastructure of chromosomes and arrests mitosis by destroying spindle fibres. Mole-Bajer (1969) found that immediately after chloral hydrate treatment in *Haemanthus katherinae*, the kinetochore and continuous microtubules were absent, but microtubules soon began to re-form anew. Since most of these studies were not quantitative, the chloral hydrate effect has mainly been described qualitatively.

The simple eukaryote *Aspergillus nidulans* has certain advantages for the study of mitosis, the most important of which is that the genetics of the organism are well known. The cytology of mitosis in *A. nidulans* is similar to that of the higher eukaryotes in most respects (Robinow & Caten, 1969). During mitosis, the chromosomes condense, a mitotic spindle is formed, the chromosomes undergo anaphase separation, the spindle then disappears, the chromosomes relax and two daughter nuclei are formed (Robinow & Caten, 1969). As with many other fungi, the nuclear membrane remains intact during all phases of mitosis. A set of temperature-sensitive, conditionally lethal, mitotic mutants of *A. nidulans* has been isolated (Morris, unpublished observations). One of these mutants has been used as a tool to analyse quantitatively the effect of chloral hydrate on mitosis.

METHODS

The wild-type haploid strain of *A. nidulans*, FGSC154 (Fungal Genetics Stock Center, Humboldt State College, Arcata, California, U.S.A.), and a temperature-sensitive (*ts*) diploid derivative derived in this laboratory from FGSC154, *ts*-706 diploid, were used for these experiments. *Aspergillus nidulans* strain *ts*-706 is characterized by a reversible anaphase arrest such that mitotic spindles and condensed chromatin accumulate co-ordinately with time at temperatures above 38 °C (Morris, unpublished). A diploid strain of *A. nidulans* *ts*-706 was constructed (Morris, unpublished) with larger, more easily identifiable spindles than the haploid. Czapek-Dox minimal medium (Difco) was supplemented with the nutritional requirements of strain 154, which were (per litre distilled water): 20 mg adenine, 0.05 mg biotin, 0.25 g methionine, 0.25 mg choline, 0.25 mg nicotine, 0.5 g sulphite and 0.5 g nitrite.

RESULTS AND DISCUSSION

Strains of *A. nidulans* 154 and *ts-706* diploid were grown in streaks on a single piece of dialysis tubing overnight at 32 °C on supplemented Czapek-Dox minimal agar medium. They were placed in liquid Czapek-Dox medium at 32 °C containing 0.02 M-chloral hydrate (Fisher, No. 723811) and samples were removed, fixed and stained at intervals. The inter-phase nuclei stain very lightly with 'aceto-orcein', while the condensed chromatin of the mitotic figures stains darkly. Acid fuchsin stains the mitotic spindle, if present, and the nucleolus (Robinow & Caten, 1969). Chloral hydrate caused the rapid disappearance of spindles such that after 15 min of exposure, none of the hyphal tips contained mitotic spindles in both strain 154 and *ts-706* diploid. In addition, chloral hydrate caused a dramatic change in the structure of the nuclei. After 20 min of exposure, more than 90 % of the hyphal tips contained nuclei in which the chromatin was darkly stained and dispersed, as opposed to the well-defined structure of the chromatin of untreated nuclei. This phenomenon may be attributed to condensed chromosomes scattered about the hyphae as in c-mitosis (Levan, 1938), or represent remnants of fragmented nuclei. With lower concentrations of chloral hydrate, these effects were observed after longer exposure times; higher concentrations of chloral hydrate produced these effects more rapidly.

Samples of strain 154 were grown overnight at 32 °C on Czapek-Dox agar media on dialysis tubing. Four pieces of tubing were transferred for 30 min into Czapek-Dox medium with 0.02 M-chloral hydrate at 32 °C, while four samples were maintained in Czapek-Dox media at 32 °C as a control. All samples were washed for 5 min in Czapek-Dox medium and returned to Czapek-Dox agar medium. Since there was no difference in the number or size of the colonies of the different samples, the chloral hydrate effect is reversible.

The *A. nidulans* strains *ts-706* (diploid) and 154 (as a control) were grown overnight and maintained at a restrictive temperature of 40 °C for 90 min until 51.5 % of the hyphal tips of *ts-706* diploid contained spindles. Each piece of dialysis tubing was divided in two such that each half contained cultures grown under identical conditions. One-half was placed in Czapek-Dox medium containing 0.02 M-chloral hydrate, at a permissive temperature of 32 °C, while the other half was placed in Czapek-Dox medium at permissive temperature as a control. The percentage of hyphal tips with mitotic spindles in *ts-706* (diploid) dropped from 51.5 to 10 % in 1 h at permissive temperature. [However, in the presence of 0.02 M-chloral hydrate, the percentage of hyphal tips with spindles dropped from 51.5 to 1 % in 15 min and to 0 % in 1 h. This shows that chloral hydrate immediately causes the disappearance of accumulated mitotic spindles.

Chloral hydrate also effectively prevents the formation of mitotic spindles. *Aspergillus* strains 154 and *ts-706* (diploid) were grown overnight at 32 °C. These cultures were transferred to Czapek-Dox media at 38 °C for 2.75 h. As described earlier, each culture was then divided in half. One-half was transferred to Czapek-Dox medium with 0.02 M-chloral hydrate at 38 °C and one-half was maintained in Czapek-Dox medium at 38 °C. While the percentage of hyphal tips with spindles increased from 25.3 to 49 % in 1 h in Czapek-Dox media at 38 °C, in Czapek medium at 38 °C containing chloral hydrate, the percentage of hyphal tips with spindles fell to 0.4 % in 15 min and to 0.2 % in 1 h. This shows that 0.02 M-chloral hydrate effectively prevents the formation of mitotic spindles, in addition to destroying those previously formed.

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