RELEASE OF DNA BREAKDOWN PRODUCTS INTO THE CULTURE MEDIUM OF *STYLONYCHIA MYTILUS* EXCONJUGANTS (PROTOZOA, CILIATA) DURING THE DESTRUCTION OF THE POLYTENE CHROMOSOMES

DIETER AMMERMANN. From the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302. Dr. Ammermann's permanent address is Zoologisches Institut der Universitaet, Tuebingen, West Germany

In *Stylonychia mytilus*, the reconstruction of the new macronucleus after conjugation occurs in two phases. Initially, the macronuclear anlage becomes polyploid and polytene giant chromosomes are visible in the nucleus. Subsequently, over 90% of the DNA is lost from the anlage which becomes diploid again. After a long "DNA-poor stage" a second polyploidization stage occurs and the macronuclear anlage develops to the definitive macronucleus (Ammermann, 1965). Scintillation-counter measurements showed that the DNA which leaves the anlage at the end of the giant chromosome stage does not stay in a TCA-insoluble form in the cytoplasm (Ammermann, 1968). However, it was not clear whether the DNA breakdown products remained in the cytoplasm in a TCA-soluble form or were released into the surrounding culture fluid.

This question was resolved with a windowless gas flow counter. The DNA of the macronucleus anlage was labeled by adding 400 μc/ml thymidine-^{3}H (Nuclear-Chicago Corp., Des Plaines, Ill. spec. act., 17, 2 mc/mM) to a culture of exconjugants during the first polyploidization stage (0–22 hr after separation of the conjugation pairs). After this, the exconjugants were washed three times with culture fluid, placed for 1 hr in fresh culture fluid, followed by 1 hr in culture fluid with 1 mg/ml cold thymidine added. After additional washing, 60 groups of 40 exconjugants (now with giant chromosomes in their macronucleus anlagen) were isolated each with 1 ml of culture fluid into 60 planchets. Ten planchets were dried immediately (25-hr value; see Fig. 1). 5 hr after this, the culture fluid of ten other planchets was removed with a pipette into ten new planchets, the animals were washed with water, and this water was added to the culture fluid; then the animals and the fluid were dried. The remaining 40 groups of animals were treated later in the same manner (see Fig. 1).

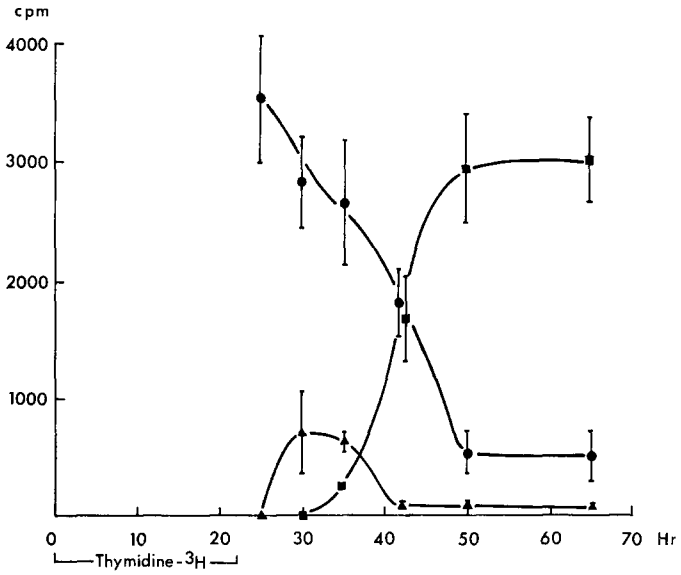

FIGURE 1 Distribution of radioactivity of *Stylonychia mytilus* exconjugants. Each value represents 10 samples of 40 exconjugants each. The time of treatment with thymidine-^{3}H is indicated; ●——● = TCA-insoluble radioactive substances in the animals; ▲——▲ = TCA-soluble radioactive substances in the animals; ■——■ = radioactivity in the culture fluid. *cpm*, counts per minute.

The dried exconjugants in the planchets were washed twice for 10 min each each with 5% TCA at 0°C. This TCA was also placed on planchets and dried. The contents of all planchets were spread with formic acid, and the radioactivity was measured in a windowless gas flow counter.

Fig. 1 shows that at the end of the first polyploidization stage, when giant chromosomes fill the macronucleus anlage (25 hr), no pool of TCA-soluble thymidine-^{3}H or their derivates exist in the exconjugants. At the end of this stage, when the DNA leaves the anlage, a large amount of TCA-soluble radioactive substances appears in the cytoplasm. Soon after this, the amount of TCA-soluble material in the cytoplasm decreases, and most of the radioactive material is found in the culture fluid. At the end of the DNA-poor stage, shortly before the second polyploidization stage (65–70 hr), about 85% of the radioactivity is in the culture fluid and very little TCA-soluble radioactive material is found in the exconjugants.

The relative amount of radioactivity given up into the culture fluid varies. In another experiment, only 75% of the radioactivity was released into the culture medium. This value probably depends on the amount of radioactivity incorporated into protein and starch, rather than into DNA (for discussion see Ammermann, 1968).

An attempt was made to determine whether or not the radioactive DNA breakdown products in the culture fluid are DNA-precursors, such as thymidine. *Stylonychia mytilus* incorporates DNA-precursors as thymidine-^{3}H essentially only in the replication bands of the macronucleus (unpublished data). In an experiment, the DNA of the macronucleus anlage of several hundred exconjugants was labeled as described above. After the breakdown of the DNA, the culture fluid was removed and added to a group of animals with replication bands. 2 hr later, radioautographs of these animals were made. They showed that the cytoplasm was labeled, but no label was visible in the replication bands. This demonstrates that the breakdown products of the DNA released into the culture fluid are not DNA-precursors but smaller fragments.

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REFERENCES

AMMERMANN, D. 1965. *Arch. Protistenk.* **108**:109.

AMMERMANN, D. 1968. *Chromosoma.* **25**:107.