SYNTHESIS OF RNA IN t^{12}/t^{12} MOUSE EMBRYOS

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An allele, t^{12} , of the multiple allelic *T*-locus is homozygous lethal to cleavage-stage mouse embryos (Smith, 1956). Other recessive alleles at this locus cause developmental abnormalities and death at specific later stages of embryogenesis (Dunn, 1956, 1964). The observations that all t^n alleles combine with *T* (as T/t^n) to produce tailless mice (Bennett, 1964) and that many of these alleles, including t^{12} , are partially complementary when combined as t^x/t^y suggest that there is a common basis for the various defects caused by *T*-locus alleles.

It has been suggested that the lethality of the t^{12}/t^{12} genome is related to abnormal nucleolar development and reduced ribosomal RNA (rRNA) synthesis at the late morula stage (Smith, 1956; Mintz, 1964). Recent studies of the homozygous t^{12} embryos, however, revealed that their definitive nucleoli differentiate normally up to the stage of developmental arrest (Hillman, Hillman & Wileman, 1970; Hillman, 1972). Condensed rounded nucleoli and abnormal labelling patterns are found in those t^{12}/t^{12} embryos which are in advanced stages of degeneration. These findings suggest that t^{12} homozygotes are not deficient in rRNA synthesis before death. The present biochemical analyses of t^{12}/t^{12} embryos were undertaken in order to resolve the discrepancy between these various findings.

In these studies, the total uptake of [^3H]5-uridine, the rates of RNA synthesis (rates of accumulation of labelled RNA), and the types of RNA synthesized during both short and long labelling periods were separately determined for embryos obtained from both $+/+$ and $+/+t^{12}$ *inter se* matings. Two-cell embryos were obtained from the oviducts of females which had been induced to superovulate (Edwards & Gates, 1959). These embryos were placed into culture (Brinster, 1963) and allowed to develop to specific cleavage stages. It should be noted that superovulation does not alter the $1(+):1(t^{12})$ transmission ratio of the $+/t^{12}$ females and that approximately 38% of such embryos of pooled litters from $+/t^{12}$ *inter se* matings are homozygous for the t^{12} allele (Hillman *et al.*, 1970). This high percentage of homozygous mutant embryos from heterozygous matings is the result of the high transmission ratio of the t^{12} allele by the $+/t^{12}$ male (Smith, 1956).

For the first series of experiments, in which both uridine uptake and rates of RNA synthesis were determined, four-cell, eight- to sixteen-cell (early morula) and sixteen- to thirty-two-cell (late morula) embryos were incubated

in [^3H]5-uridine (6 $\mu\text{Ci/ml}$, 20 to 25 Ci/mmol, Schwarz) for 3 hr. Following this treatment, the embryos were washed four times in non-radioactive medium and frozen at -70°C . A perchloric acid-ribonuclease microextraction procedure (Tasca & Hillman, 1970) was used in order to obtain the uridine uptake and RNA synthesis data. The rate of RNA synthesis was expressed as percentage incorporation (RNA fraction/total uptake of precursor $\times 100$). The latter expression was adopted when it was first noted that the uptake of [^3H]5-uridine into cleavage-stage mouse embryos increases rapidly after the four-cell stage (Tasca & Hillman, 1970; Daentl & Epstein, 1971). The rates of uridine uptake and rates of RNA synthesis in embryos from $+/+$ *inter se* matings were compared with these same processes in pooled litters from $+/t^{12}$ *inter se* matings. Since the $+/t^{12}$ litters contain 38 to 40% t^{12}/t^{12} embryos, a reduction in either uptake or synthesis between these litters should be evident. Each experiment was repeated at least three times.

In the second series of experiments, pooled litters from both $+/t^{12}$ and $+/+$ *inter se* matings were separately placed into culture at the two-cell stage. These embryos were either allowed to develop to the late morula stage when they were treated with 6 μCi [^3H]5-uridine/ml medium for 3 hr, or they were placed into this labelled medium at the two-cell stage and allowed to develop to the late morula-early blastocyst stage (about 45 hr). In both studies, the embryos were removed from the medium when a cavity had developed in approximately 30 to 40% of them. Since only a small percentage of t^{12}/t^{12} embryos grow to the blastocyst stage (Hillman *et al.*, 1970), the 30 to 40% of the embryos from $+/t^{12}$ *inter se* matings which develop a cavity should be composed almost exclusively of $+/+$ and $+/t^{12}$ embryos, while 57 to 67% of those which did not develop a cavity should be of the homozygous t^{12} genotype. In these experiments, therefore, differences in RNA synthesis between samples composed of over 50% late morula t^{12}/t^{12} embryos and samples composed entirely of control $+/+$ late morula embryos should be measurable and significant. In these studies, the embryos without a cavity were separately collected in approximately 30 μl medium and their RNA was subjected to sucrose density-gradient centrifugation (Tasca & Hillman, 1970).

In the first series of experiments, the uptake of uridine by the combined litters from $+/t^{12}$ *inter se* matings was not significantly different from the uptake of the precursor by the pooled litters from $+/+$ matings (Text-fig. 1). In both groups, there was a threefold increase in uptake between the four-cell and eight- to sixteen-cell stages and a greater than fourfold increase between the latter stage and the late morula stage.

Synthesis of RNA, expressed as percentage incorporation, increased progressively from about 17% at the late four-cell stage to about 30% at the late morula stage in both the pooled litters containing homozygous mutant embryos and the wild-type litters (Text-fig. 1). The latter experiments show that litters composed of 40% t^{12}/t^{12} embryos did not differ significantly from litters composed entirely of $+/+$ embryos in the percentage incorporation of [^3H]5-uridine into RNA at any stage examined.

In the second series of experiments, the data from the 3-hr labelled morulae were separately obtained and then plotted on the same graph (Text-fig. 2),

which shows that the three major RNA species were synthesized by both the mutant late morulae (sample containing at least 50% t^{12}/t^{12} embryos) and by the $+/+$ late morulae. In both late morulae groups, there were sharp coincident radioactive peaks in the 4S, 18S and 30 to 32S regions. The data show that there were no differences between control and mutant embryos in the synthesis of 4S, 18S and 30 to 32S RNAs relative to the overall rate of RNA synthesis. The usual 2:1 ratio between the two major ribosomal RNA peaks was observed and the rate of ribosomal synthesis was 60 to 70% of the overall rate of RNA

TEXT-FIG. 1. The rate of uptake of [^3H]5-uridine and rate of synthesis of RNA (percentage incorporation) by $+/+$ and t^{12}/t^{12} mouse embryos at specific cleavage stages. Embryos were labelled for 3 hr at each stage. In all experiments, the age of the embryos was determined by cell counts on randomly selected samples. (Perchloric acid-ribonuclease fractionation procedure).

synthesis. The rate of 4S RNA synthesis was 7 to 11% of the overall rate of RNA synthesis (in close agreement with data of Ellem & Gwatkin, 1968) and was consistently found to be 30 to 40% of the rate of synthesis of 18S ribosomal RNA.

The data from the 45-hr labelling experiments indicate that there were no detectable differences in the RNA species or relative amounts of RNA synthesized and accumulated by the t^{12}/t^{12} and control embryos (Text-fig. 3). The ribosomal RNAs with the larger species sedimenting at 28S had the same approximate 2:1 relationship to each other as in the short labelling experiment but represented a larger percentage of the overall rate of RNA synthesis (75%

TEXT-FIG. 2. Synthesis of RNA by $+/+$ and t^{12}/t^{12} late morula (LM) mouse embryos incubated in $[^3\text{H}]$ 5-uridine for 3 hr. (Sucrose density-gradient analysis). The sample labelled t^{12}/t^{12} is composed of at least 57% recessive homozygotes (see text).

to 85%) than in the 3-hr period. The 4S region represented about 7% of the total rate of RNA synthesis and no longer appeared as distinct a peak as in the short labelling experiments, suggesting the possible accumulation of stable 5S RNA (Woodland & Graham, 1969). As in the case of the 3-hr labelling experiment, synthesis of molecules in this light region was 30% to 40% of the rate of synthesis of 18S rRNA. The increased absolute amount of RNA accumulation by the mutant embryos may reflect a decrease in turnover of RNA as these embryos approach the lethal period.

The data indicate that t^{12}/t^{12} embryos are capable of normal rates of uridine transport and utilization for RNA synthesis. The relative rates of synthesis of 18S to 30 and 32S rRNA were the same in both the mutant and control late morulae. The observed relationship of the rate of synthesis of 4S RNA to 18S rRNA was also present in both genotypes. With the previous autoradiographic observations (Hillman, 1972), these results strongly suggest that RNA synthesis and processing proceed at normal rates in t^{12}/t^{12} embryos. Thus, the hypothesis that t^{12}/t^{12} embryos are defective in nucleolar function becomes much less tenable in view of (1) evidence from the present work, (2) the finding

TEXT-FIG. 3. Synthesis of RNA by +/+ and t^{12}/t^{12} late morulae (LM) mouse embryos incubated in [^3H]5-uridine for 45 hr (from two-cell to late morula stage). (Sucrose density-gradient analysis.) The sample labelled t^{12}/t^{12} is composed of at least 57% recessive homozygotes.

that the numbers of nucleoli in mouse cells are not determined by a single major gene (Ivanyi, 1971; Flaherty, Bennett & Graef, 1972), (3) the fact that the ninth linkage group in the mouse does not contain a secondary constriction (Bennett, 1965), and (4) the observation that late morulae t^{12}/t^{12} embryos do not differ in their intensity of Azure-B stain when compared with +/+ late morulae (Calarco & Brown, 1968).

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