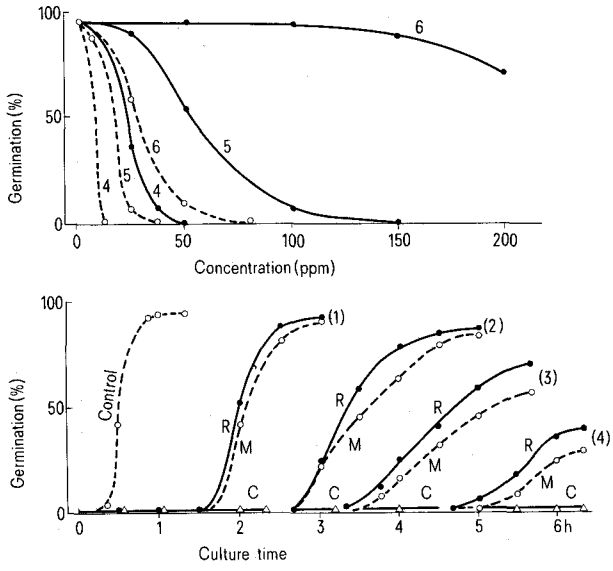

Fig. 2. Effects of royal jelly acid and myrmicacin on germination of *Camellia sinensis* pollens (–●– royal jelly acid, –○– myrmicacin); top chart: relations of agent concentration to germination percentage (numbers indicate culture pH); bottom chart: change of germination percentage of inhibited pollens after transfer to agent-free cultures; R: royal jelly acid (100 ppm), M: myrmicacin (50 ppm), C: capric acid (50 ppm), Control: agent-free medium (pH 5.5), numbers in parantheses indicate period of inhibition in h.

inhibition, and culture on the agents over 6 h brought no germination.

In our previous paper[6], we pointed out the essential structure of a terminal carboxyl group and of normal chain with 8 through 10 carbon atoms for the inhibitory activities of myrmicacin and analogous compounds. From a consideration of our previous and present experimental results, it seems reasonable to assume that release from the inhibition yielded with the agents is concerned with the presence and position of hydroxyl group in the agent molecules. These natural inhibitors used by the insects should also be able to be employed as 'soft inhibitors' in various biological researches.

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Globin gene expression in MSV-transformed fibroblasts

I. Parker and W. Fitschen[1]

Department of Biochemistry, University of Cape Town, Rondebosch 7700 (Republic of South Africa), 28 December 1978

Summary. The activation of globin gene expression on viral transformation of 3T3 cells was investigated. Globin mRNA was determined using a radioactive complementary DNA probe. No difference was found between 3T3 and transformed 3T3 cells. There does not therefore appear to be a random activation of extensive regions of the cellular genome.

Transformation of 3T3 mouse embryo fibroblasts with mouse sarcoma virus (MSV/3T3) results in loss of contact inhibition, and a rapid increase in cell growth rate as well as cell density, without the production of active virus, type C particles or MSV gene products[2]. The MSV genome has been shown to remain in a stable heritable form for more than 100 generations[3].

Groudine and Weintraub[4] detected the presence of 100–500 copies of fetal globin mRNA sequences per cell in Rous sarcoma virus transformed chicken embryo fibroblasts (RSV/CEF), whereas in cells infected with a mutant carrying a deletion of the 'onc' gene (believed to be responsible for the phenotypic changes that occur on viral transformation), transcription of globin genes did not occur[4]. In 3T3 cells, very low levels of globin mRNA sequences are present in polysomes, but no haemoglobin is produced[5].

An investigation of globin mRNA metabolism in 3T3 and MSV/3T3 cells may help towards understanding the basic differences in the molecular events between normal and transformed cells, especially with regard to random versus specific gene activation. In our experiments, we found no increased levels of globin mRNA sequences in either the nuclear or cytoplasmic compartments of MSV/3T3 cells.

Materials and methods. AMV reverse transcriptase was supplied by Dr J.W. Beard (Life Sciences Inc., Florida, USA); (^{3}H)-dCTP was obtained from Radiochemical Centre, Amersham; Oligo $d(T)_{12-18}$, Oligo d(T) cellulose, S_1 nuclease and DNase from Miles Laboratories, deoxyribonucleoside triphosphates from Boehringer, Mannheim (Federal Republic of Germany); and 3T3 cells from Flow Laboratories. MSV/3T3 cells were a gift from Dr E.L. Wilson (Clinical Science and Immunology, University of Cape Town).

Isolation of globin mRNA. ICR/HA mice were made anemic by s.c. injections of 0.6 ml per 100 g body mass of a 0.25% solution of phenylhydrazine chloride in saline. Blood was collected by cardiac puncture, and RNA isolated from washed red blood cells as described by Lanyon et al.[6]. The poly A containing RNA (mRNA) was separated by Oligo d(T) cellulose chromatography[7]. Such RNA preparations have been shown to stimulate the synthesis of globin in an ascites cell free extract (D.E. Woods, unpublished).

Polyacrylamide gel-electrophoresis. Electrophoresis of RNA on 2.6% polyacrylamide gels was as described by Loening[8]. RNA samples were dissolved in electrophoresis buffer containing 0.5% SDS, heated to 60 °C for 10 min, chilled rapidly and applied to the gels. Electrophoresis was for 90 min at 5 mA per gel. The markers used were 4S yeast tRNA and 5S, 18S and 28S ribosomal RNA.

cDNA synthesis. (^{3}H)-cDNA complementary to mouse glo-

bin mRNA was synthesised as described by Harrison et al.[9] and modified by Woods and Fitschen[10], except that the final concentration of (^{3}H)-dCTP was 1 mCi/ml, and the incubation volume was 50 µl. The specific activity of the cDNA was 3.153×10^7 dpm/µg.

RNA:cDNA hybridizations. Hybridization reactions were carried out in 10 µl volumes in sealed siliconized capillary tubes. Analysis of the hybrids by S_1 nuclease digestion was done as described by Gilmour et al.[11].

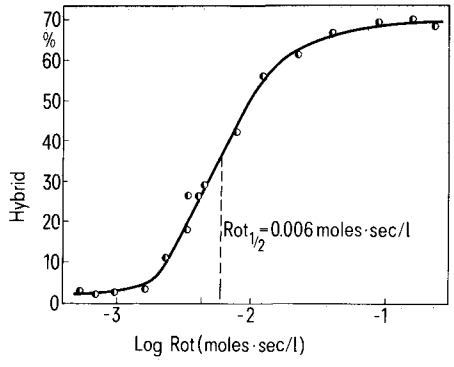

Fig. 1. Kinetics of reannealing of globin cDNA to globin mRNA. Hybridizations were done using 0.2 ng cDNA per 10 µl incubation. The mRNA concentration varied between 0.05 and 5 µg/ml, and the time of incubation between 1 and 10 h, to obtain the Rot values indicated.

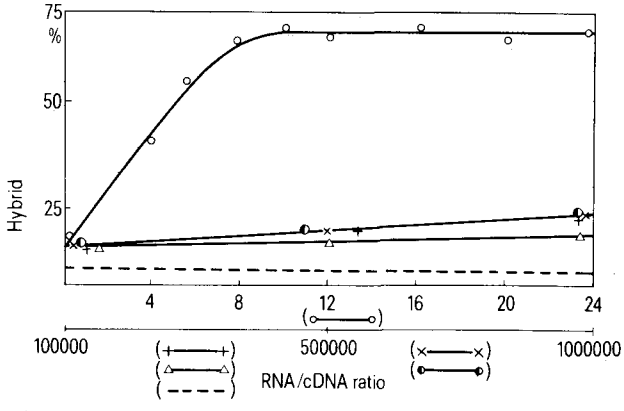

Fig. 2. Titration of globin cDNA to globin mRNA and to RNA from 3T3 and MSV/3T3 cell fractions. O–O: globin mRNA, △–△: MSV/3T3 nuclear RNA, ◐–◐: MSV/3T3 cytoplasmic RNA, +–+: 3T3 nuclear RNA, ×–×: 3T3 cytoplasmic RNA and ---: *E. coli* RNA. Reaction mixtures contained 0.05 ng cDNA incubated to a Cot of 0.06 moles · sec/l (14 days), with increasing amounts of RNA.

Cell cultures and RNA extraction. 3T3 cells were grown in 75 cm^2 Falcon flasks in Hanks minimal essential medium containing 1% fetal calf serum. Cells were harvested with a rubber policeman and washed in ice-cold phosphate buffered saline. The cells were then pelleted and suspended in 10 volumes of RSB buffer (10 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$) containing 0.5% Triton X-100, and lysed by homogenizing with 20 strokes of the tight pestle in a Dounce homogenizer. Nuclei were pelleted at $1000 \times g$ for 4 min, washed thrice with RSB buffer, and deproteinized with phenol:chloroform (1:1), using the method of Perry et al.[12]. The DNA was digested with RNase free DNase as described by Stein et al.[13].

The mitochondria were pelleted at $16{,}000 \times g$ for 15 min and total cytoplasmic RNA was extracted from the postmitochondrial supernatant as described by Perry et al.[12].

Results and discussion. Polyacrylamide gel-electrophoretic analysis of the globin mRNA revealed a major peak with sedimentation coefficient of 9S, and caused 70% of the cDNA probe to become hybridized. Background hybridization in the absence of globin mRNA was 2–7%. The kinetics of hybridization between the cDNA/mRNA is shown in figure 1. The reaction proceeded with a $Rot_{1/2}$ value of 0.006 moles · sec/l, which is in good agreement with those of other workers[14,15]. When globin cDNA is titrated to increasing amounts of RNA, as little as 5×10^{-5}% mRNA sequences may be detected with a probe of similar specific activity[5].

When the 3T3 and MSV/3T3 nuclear and cytoplasmic RNA was hybridized to the globin cDNA probe, only 16–20% of the cDNA was double-stranded at a RNA:DNA ratio of 10^6:1, as shown in figure 2. These incomplete titration curves show that approximately 10^{-4}% of these RNA species consist of globin mRNA. That this low extent of hybridization is not nonspecific is shown by the fact that *Escherichia coli* RNA hybridizes maximally to only 7% even at a RNA:DNA ratio of 2×10^6:1. The level of globin mRNA in MSV/3T3 nuclear RNA appears to be lower than in the nuclear RNA from untransformed cells, however the difference is insignificant.

Our results showed that there is no increase in globin mRNA levels in MSV/3T3 cells, as reported for RSV/CEF. The increase in abundance of poly (A) containing RNA on the polysomes of MSV/3T3 cells observed by Rolton et al.[2] is therefore not likely to be due to random activation of gene transcription. These increased RNA species could possibly be coding for growth factors released by these cells into the medium, as no growth factors are released by their untransformed counterparts[16].

1 We wish to thank Dr J.W. Beard for supplying the AMV reverse transcriptase. This work was supported by grants from the CSIR and the University of Cape Town Research Committee. Present address of W.F.: Department of Medical Biochemistry, University of the Witwatersrand, Medical School, Johannesburg 2001, S.Africa.

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