INITIATION OF DNA SYNTHESIS IN RAT THYMUS: CORRELATION OF CALCIUM-DEPENDENT INITIATION IN THYMOCYTES AND IN ISOLATED THYMUS NUCLEI*

Leigh A. Burgoyne, M. Anwar Waqar and Maurice R. Atkinson
School of Biological Sciences, Flinders University of South Australia, Bedford Park, South Australia 5042.

Received April 21, 1970

SUMMARY: Initiation of DNA synthesis in nuclei isolated from rat thymus is stimulated by Ca^{2+}, as shown in previous studies with liver nuclei. 3',5'-Cyclic AMP did not replace Ca^{2+} in either system. The relationship of this Ca^{2+}-dependent stimulation to published reports of Ca^{2+}-dependent promotion of mitosis in vivo and to Ca^{2+} stimulation of initiation of DNA synthesis in isolated thymocytes, is discussed.

Raising Ca^{2+} levels in the blood stimulates mitotic activity in rat thymus; and in studies with isolated rat thymocytes, exposure to 1-2 mM Ca^{2+} promotes initiation of DNA synthesis (for a review, see Whitfield et al.; Ref. 1). It has recently been shown (2) that rat liver nuclei, isolated in the presence of EDTA and EGTA[1], are unable to initiate DNA synthesis until their DNA has been nicked in a Ca^{2+}-dependent reaction. Whitfield et al. (1) have discussed the possibility that changes in intracellular Ca^{2+} levels may be involved in physiological control of DNA replication and cell division. It has also been suggested that stimulation of DNA synthesis and mitotic activity in thymocytes by Ca^{2+} might involve increased formation of 3',5'-cyclic AMP (3).

To find if the Ca^{2+} effect described for isolated liver nuclei was relevant in thymus, where comparison with extensive physiological studies on DNA replication (1) is possible, the involvement of Ca^{2+} in initiation of DNA synthesis in isolated thymus nuclei has now been studied. As with liver nuclei, Ca^{2+} promoted nicking of DNA and permitted initiation of DNA synthesis; no significant effect of cyclic AMP could be shown.

Methods - Nuclei were isolated from rat thymus in the presence of EDTA and EGTA as described for rat liver nuclei (2), and were not activated with Ca^{2+} before assay.

---

* This work was supported by grants from the Australian Research Grants Committee and the University of Adelaide Anti-Cancer Foundation.

1 EGTA : Ethylene glycol-bis-(2-aminoethyl ether)-N,N'-tetraacetic acid.

DNA synthesis was measured at 37° as described before (2), in the absence of Ca^{2+} or with added Ca^{2+} as indicated in figure legends.

In attempts to detect an effect of cyclic AMP on initiation or extent of DNA synthesis, assays were carried out in the presence of this nucleotide at concentrations in the range 10^{-9}M - 10^{-4}M. In these assays incorporation of label from α-^{32}P-dTTP in the presence of phosphoenol pyruvate, ATP, dATP, dCTP and dGTP (2) was followed over periods up to 80 min.

Labeled DNA was centrifuged in alkaline sucrose gradients and the distribution of ^{32}P-DNA and total DNA was measured (cf. 2).

Results and Discussion - Thymus nuclei isolated in the presence of EDTA and EGTA contain DNA polymerase that incorporates labeled deoxyribonucleotide in the presence of Mg^{2+} and absence of Ca^{2+} when poly d(A-T) is provided as template/

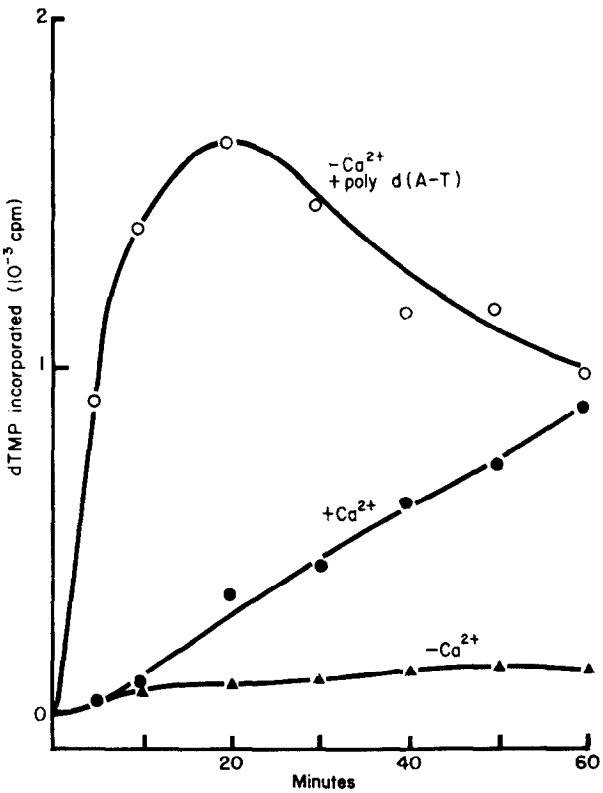

Figure 1. Incorporation of α-^{32}P-dTMP by thymus nuclei in buffer A (2) containing 0.34 M sucrose, 2 mM phosphoenol pyruvate, 0.4 mM each of ATP, dATP, dCTP, dGTP, 20 µM α-^{32}P-dTTP (7.6 x 10^8 cpm/µmole), 10 mM $MgCl_2$, 1 mM EDTA and 0.2 mM EGTA. Each assay (50 µl) contained nuclei corresponding to 0.14 µmole of DNA-P. Incorporation was measured: in the absence of Ca^{2+}, —▲— ; in the absence of Ca^{2+}, but with poly d(A-T), 60 µM in residues, —○— ; with no poly d(A-T), but with 1 mM $CaCl_2$, —●— .

primer (Fig. 1). With no added primer or Ca^{2+}, incorporation of label into nuclear DNA was near the lower limit of detection. However, as found with liver nuclei (2), there was a rapid and sustained synthesis of DNA, without added primer, when nuclei were incubated with deoxyribonucleoside triphosphates in the presence of 1 mM $CaCl_2$ (Fig. 1). In contrast to the results with rat liver nuclei (2), net synthesis primed by poly d(A-T) reached a maximum and then decreased during the assay period, indicating the presence of degradative enzymes.

The dependence of thymus nuclear DNA synthesis on the concentration of added $CaCl_2$ was studied (Fig. 2). Maximal synthesis during 30 min. was found with 2 mM $CaCl_2$, and half-maximal stimulation with 1 mM $CaCl_2$, but significant stimulation of synthesis was consistently found at $CaCl_2$ levels as low as 0.1 mM, even though the system contained 1 mM EDTA and 0.2 mM EGTA. The apparent activity of Ca^{2+}, measured with a Ca^{2+} electrode and corrected for Mg^{2+} interference, was approximately 30 µM in conditions of half-maximal activation. In studies on initiation of DNA synthesis in isolated thymocytes (1), half-maximal stimulation of DNA synthesis was found at 1 mM external Ca^{2+}.

In the liver nuclei studied previously (2) evidence was given that Ca^{2+}

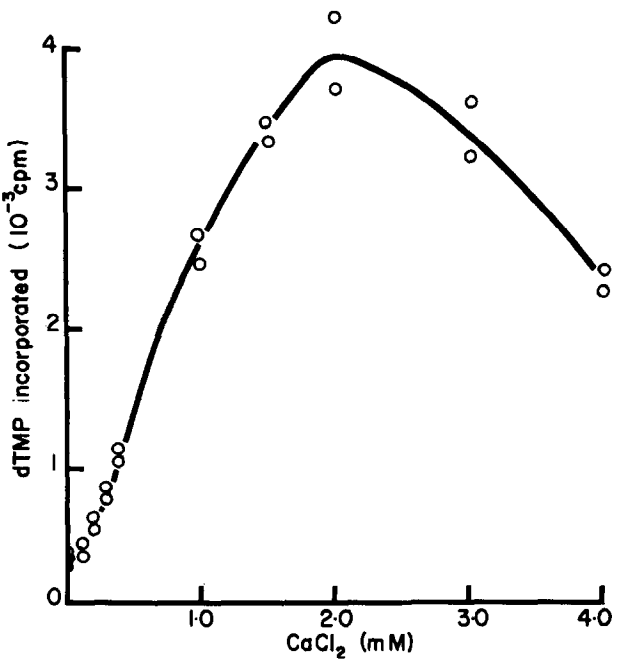

Figure 2. Dependence of DNA synthesis on added $CaCl_2$ in thymus nuclei. Nuclei (0.34 µmole DNA-P in 50 µl of assay solution) were incubated for 30 min, as in Fig. 1, with no added poly d(A-T) and with $CaCl_2$ at the concentrations shown. Incorporation of label from α-^{32}P-dTTP (7.0 x 10^8 cpm/µmole) in replicate assays is shown.

activates a system that nicks DNA and permits priming of DNA synthesis. Centrifugation in an alkaline sucrose gradient (Fig. 3) shows that Ca^{2+} also promotes nicking of DNA in thymus nuclei, and that the labeled DNA (8S - 13S) is smaller than the bulk DNA. The marked Ca^{2+} dependence of initiation of DNA synthesis in thymocytes (1) correlates with the requirement for Ca^{2+} in initiation of DNA synthesis in isolated thymus nuclei, and this raises the possibility that a Ca^{2+}-dependent endonuclease nicks the thymus DNA and may have a physiological role in control of DNA replication.

There is increasing evidence that a number of regulatory effects often attributed to intracellular cyclic AMP may more directly involve changes in intra

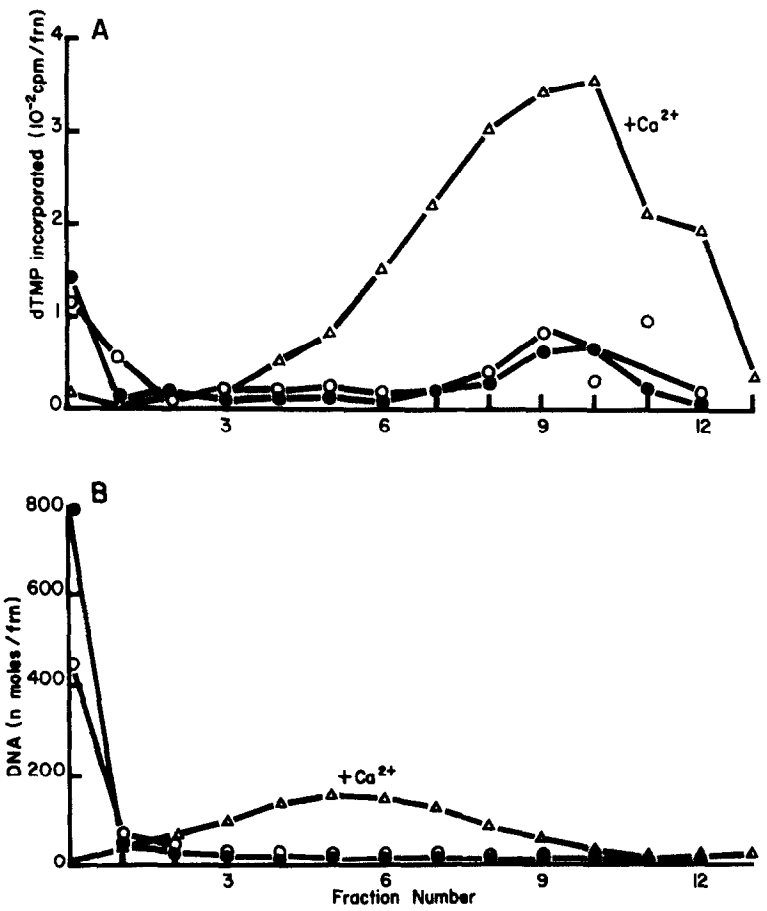

Figure 3. Thymus nuclei (1.1 µmoles DNA-P in 100 µl of assay solution) were incubated for 30 min. as in Fig. 1; the specific activity of the dTTP was 2.4 x 10^8 cpm/µmole. Distribution of labeled DNA (A) and total DNA (B), after centrifuging in an alkaline sucrose gradient, was measured as described before (2). Incubation in the presence of 1 mM $CaCl_2$, -△- ; 10^{-4}M cyclic AMP but no added $CaCl_2$, -○- ; no $CaCl_2$ or cyclic AMP, -●- .

cellular activity of Ca^{2+} (for a recent review see Nagata and Rasmussen; Ref. 4). It is not known if the effect of external Ca^{2+} on DNA synthesis in thymocytes involves changes in intracellular cyclic AMP concentrations (cf. 1,3), but cyclic AMP does not replace Ca^{2+} in promoting DNA synthesis in isolated thymus nuclei. Assays containing 10^{-9} - 10^{-4} M cyclic AMP did not differ significantly from the "-Ca^{2+}" control in Fig. 1. Cyclic AMP in this range also had no significant effect on initiation of DNA synthesis in liver nuclei isolated in Ca^{2+}-free conditions, or on the rate of DNA synthesis in liver nuclei activated (2) by exposure to Ca^{2+}. These negative results are not shown, but a sucrose gradient analysis of DNA from thymus nuclei incubated with labeled nucleotide in the presence of 10^{-4} M cyclic AMP (Fig. 3) shows a distribution of labeled DNA and total DNA similar to that in the control with no added Ca^{2+} or cyclic AMP.

If operation of a Ca^{2+}-dependent endonuclease is involved in control of nuclear DNA synthesis it is difficult to envisage this mechanism accounting for the specificity required for sequential initiation in eukaryotic replicons. Investigation of the Ca^{2+} dependence as one component of the replication control system may be useful in recognition of other more specific regulatory factors.

References

1. Whitfield, J.F., Rixon, R.H., Perris, A.D., and Youdale, T., Exptl. Cell Res., 57, 8 (1969).
2. Burgoyne, L.A., Waqar, M.A., and Atkinson, M.R., Biochem. Biophys. Res. Comm., 39, 918 (1970).
3. Macmanus, J.P., and Whitfield, J.F., Exptl. Cell Res., 58, 188 (1969).
4. Nagata, N., and Rasmussen, H., Proc. Natl. Acad. Sci. U.S., 65, 368 (1970).