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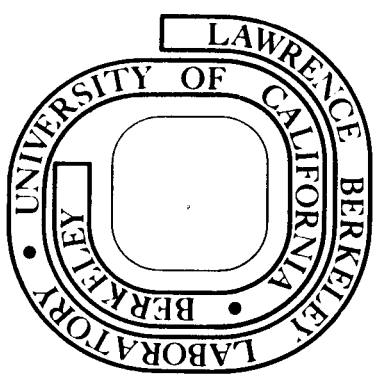

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ELEVATED RNA-INSTRUCTED DNA POLYMERASE ACTIVITY IN A NEW CELL LINE

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Summary: The new cell line, UCl-B, yields an unusually high specific activity of a viral-like RNA-instructed DNA polymerase.

A new cell line, UCl-B, has been described[1]. The new line was derived from a branch culture of standard mouse Balb/3T3 cells. Balb/3T3 cells replicate Moloney leukaemia virus (MLV) without alteration of cellular growth or morphology while UCl-B cells transform in response to MLV infection. Electron microscopy of MLV-infected UCl-B cells disclosed the presence of aberrant particles of unknown origin in addition to typical C-type particles.

In this paper we report that when infected with MLV the UCl-B cell line contains an unusually large amount of RNA-instructed DNA polymerase (RDP) activity. Aaronson's Balb/3T3 Clone A31[2] infected with murine sarcoma virus (MSV) was used for comparison. The RDP polymerase activity extracted from both types of infected cells shows the template and divalent cation responses characteristic of the virus-associated RDP, in contrast to the characteristics of E. coli DNA polymerase I.

Amount of RDP in Infected Cells

Significant levels of RDP activity were found in tissue culture cells only after viral infection. Assays for this polymerase activity in extracts

from uninfected cells, either clone A31 or UCl-B, never gave a specific activity which was greater than 1% of that found in the infected cell extracts even when subconfluent, growing cells (mitotic index $\sim$ 5%) were extracted. The absence of significant activity in the uninfected cell extract was not due to the presence of an inhibitor since addition of twice as much protein (and volume) from extracts of uninfected UCl-B cells caused very little or no inhibition of the polymerase activity extracted from MLV-infected and transformed UCl-B cells.

Very little activity was found in clone A31 or UCl-B cells during the first two days after virus infection. On the third day after infection and coincident with the appearance of infective virus and polymerase activity in the medium, significant levels of polymerase were detectable in the cells. The enzyme activity in the clone A31 cells increased with time until the 4th or 5th day post infection while activity in the UCl-B cells appeared to increase through the 6th day. RDP extractions were done on the 6th day when the cells were beginning to be released from the substrate.

When the RDP extractions were done on the 6th day post infection, the specific activity of the enzyme from MLV-transformed UCl-B cells was 10-20 fold higher than from MSV-transformed clone A31 cells and 5-10 fold higher than from MLV-infected clone A31 cells (Table I). The difference between the enzyme's specific activity in MSV-transformed and MLV-infected clone A31 cells is probably due to the fact that the MLV-infected cells are density inhibited while the MSV-transformed cells are growing and would thus be expected to have higher levels of cellular

proteins. It is important to note that the presence of the polymerase activity in the cells does not correlate with the growth phase of the cells but does correlate with the presence of infective virus.

Template Specificity

Table I also shows that the polymerase activities extracted from the two different cells lines after infection with virus and from partially purified MLV virions all have template preferences characteristic of virus-associated DNA polymerase, i.e., prefer poly-rA to poly-dA as template with oligo-dT as primer for both templates[3,4,5]. For these four sources of polymerase, the activity on poly-rA is at least 170 fold greater than that on poly-dA. The preferences for poly-rA as template is demonstrated using either Mn^{2+} or Mg^{2+}, although Mn^{2+} is the preferred ion.

E. coli DNA-polymerase I (Kornberg polymerase) was used as a DNA-dependent DNA polymerase control, and, as shown in Table I, this enzyme preferred the poly-dA as template using either Mn^{2+} or Mg^{2+}, but Mg^{2+} is here the preferred ion. The template activity ratio of poly-rA to poly-dA obtained for the E. coli DNA polymerase using Mg^{2+} and approximately equal base ratios of adenine and thymidine was 0.18 which compares very well with the ratio of 0.2 obtained for this enzyme under similar assay conditions by Robert, et al.[5] and Wells, et al.[4] Using Mg^{2+} and a 20:1 base ratio, we also obtained an activity ratio (poly-rA/poly-dA) of 0.18 which compares to the ratios of 0.3 and 1.0 obtained by Goodman and Spiegelman[3] under similar assay conditions.

The MLV-transformed UCl-B cell extract and the partially purified MLV virion extract also showed significant activity on poly-rC:oligo-dG with either Mn^{2+} or Mg^{2+}, again a characteristic of the virus-associated

RDP. In the presence of Mn^{2+}, the MLV-transformed UCl-B cell extract and the partially purified MLV extract were 18% and 12%, respectively, as active on poly-rC:oligo-dG as on poly-rA:oligo-dT. This percent activity is in very good agreement with similar observations by Baltimore and Smoler[6].

The E. coli enzyme was unable (< 1%) to use the poly-rC:oligo-dG as a template primer regardless of the cation used. Of the mammalian DNA polymerases tested none have been able to use poly-rC:oligo-dG as a template primer[7-10].

The E. coli enzyme was further assayed under conditions for assay of RNA-instructed DNA polymerase activity (conditions A) in order to be certain that the template preference of this known DNA-dependent DNA polymerase was not significantly altered by the conditions of assay. The results of one such experiment are shown in Table II. This experiment gave the greatest change in the template activity ratio, reaching 1.05 under conditions A from a value of 0.5 under more standard DNA-dependent DNA polymerase assay conditions which still used Mn^{2+} as the divalent cation (conditions B). The average template activity ratio from several such experiments was 0.82 under assay conditions A and 0.52 under conditions B. Thus, the assay conditions do slightly alter the template activity ratio for the E. coli enzyme, but this ratio is distinctly different from those ratios obtained for the RNA-instructed DNA polymerase activity from virus-infected cells which varied from 170 to 1800 (Table I). It is also interesting to note that the activity of highly purified DNA polymerase from E. coli is affected by low concentrations of Triton DN-65 especially in the presence of 100 mM KCl even to the extent of making it as responsive to an RNA template as to a DNA template.

The experiments shown in Table III were done to ensure that if a DNA-dependent DNA polymerase activity similar to the known enzyme activity from E. coli were present in significant amounts in the MLV-transformed UC1-B cell extract, such an enzyme activity would have been detected using a DNA template. By adding to the UC1-B cell extract enough E. coli polymerase to yield a 3% contamination of the activity measured on an RNA template (2.0 pmole/hr added to 67.2 pmole/hr) and then assaying this mixture on a DNA template, the activity of the added contaminant was easily detected (3.0 pmole/hr). Larger amounts of contamination were proportionally more obvious on the DNA template. If all of the UC1-B cell extract's activity on a DNA template (0.3 pmole/hr) were due to a contaminating DNA-dependent DNA polymerase similar to that of E. coli, the contaminant's contribution to the activity on an RNA template would be 0.2 pmole/hr out of 67 pmole/hr or $\sim$0.3%. Thus, from these experiments we can say that <3% (and probably <0.3%) of the activity we assay in our standard assay could be contributed by such a DNA-dependent DNA polymerase contaminant.

The MLV-infected and transformed UC1-B cells were found to be an unusually good source of the RNA-instructed DNA polymerase activity from virally-transformed cells since the enzyme appears to be present at levels which are an order of magnitude above those in other virally-transformed cells. The high enzyme level in the UC1-B cells may prove extremely useful in detecting a similar activity when these cells are transformed by chemical carcinogens or radiation.

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References

1. Hackett, A. J. and Sylvester, S. S., Nature New Biology, 239, 164 (1972).
2. Aaronson, S. A. and Todaro, G. J., J. Cell Physiol. 72, 141 (1968).
3. Goodman, N. C. and Spiegelman, S., Proc. Nat. Acad. Sci. USA, 68, 2203 (1971).
4. Wells, R. D., Flügel, R. M., Larson, J. E., Schendel, P. E. and Sweet, R. W., Biochem., 11, 621 (1972).
5. Robert, M. S., Smith, R. G., Gallo, R. C., Sarin, P. S. and Abrell, J. W., Science, 176, 798 (1972).
6. Baltimore, D. and Smoler, D., Proc. Nat. Acad. Sci. USA, 68, 1507 (1971).
7. Smith, G., and Gallo, R. C., Proc. Nat. Acad. Sci. USA, 69, 2879 (1972).
8. Bobrow, S. N., Smith, R. G., Reitz, M. S. and Gallo, R. C.,Proc. Nat. Acad. Sci. USA, 69 3228 (1972).
9. Weissbach, A., Bolden, A., Muller, R., Hanafusa, H. and Hanafusa, T., J. Virol. 10, 321 (1972).
10. Chang, L.M.S. and Bollum, F. J., Biochem. 11, 1264 (1972).
11. Ross, J., Scolnick, E. M., Todaro, G. J. and Aaronson, S. A. Nature New Biology, 231, 163 (1971).

Table I

Template Preferences

Activity Source	Cation	poly-rA:oligo-dT 0.5 µg:0.5 µg	poly-dA:oligo-dT 0.5 µg:0.5 µg	ratio rA/dA	poly-rA:oligo-dT 1.0 µg:0.05 µg	poly-dA:oligo-dT 1.0 µg:0.05 µg
MLV -transformed UCl-B cell extract	Mn^{2+}	194	0.11	1800	55.4	< 0.01
	Mg^{2+}	1.95	< 0.01	-	0.22	< 0.01
MSV -transformed clone A31 cell extract	Mn^{2+}	8.47	0.05	170	2.26	< 0.01
	Mg^{2+}	0.25	< 0.01	-	< 0.01	< 0.01
MLV -infected clone A31 cell extract	Mn^{2+}	20.8	0.02	1000	6.28	< 0.01
	Mg^{2+}	0.93	< 0.01	-	< 0.01	< 0.01
Partially purified MLV virion extract	Mn^{2+}	254	< 0.01	> 25000	114	< 0.01
	Mg^{2+}	5.02	< 0.01	-	0.54	< 0.01
Growing UCl-B cell extract	Mn^{2+}	0.02	< 0.01	-	0.01	< 0.01
	Mg^{2+}	< 0.01	< 0.01	-	< 0.01	< 0.01
E. coli DNA polymerase I	Mn^{2+}	10000	19400	0.52	5120	12700
	Mg^{2+}	10900	61300	0.18	2920	15900

Footnote to Table I

Numbers given, except for the ratio, are activity in pmole/hr per μg protein. Ratio numbers were obtained by dividing column 1 by column 2.

Assay Conditions

All assays were done in duplicate. Assays on the first five activity sources were done under assay conditions A (86 mM Tris-HCl, pH = 7.8; 0.6 mM dithiothreitol; 100 mM KCl; and 0.01% Triton DN-65). The E. coli DNA polymerase was assayed under conditions B (81 mM Tris-HCl, pH = 7.8 and 0.5 mM dithiothreitol). All assays were done in 100 μl which contained 0.02 mM [^{3}H]dTTP (1 C/mmole), 6 mM NaCl, 2% glycerol, and the divalent cation and template-primer complex indicated in the table. When present, Mn^{2+} was 0.1 mM and Mg^{2+} was 6 mM. The numbers immediately below each template-primer complex indicate the amount of each polymer present in the assay. The template-primer complexes were prepared by annealing for 5 min at 60°C with slow cooling (3 hr) mixtures containing either a 1:1 or 20:1 weight ratio of adenine to thymidine in a final combined concentration of 250 or 263 μg/ml of 0.01 M Tris-HCl, pH = 7.4, and 0.15 M NaCl.

Activity Source

The infected cells used for RDP extractions shown in Table I were seeded in 250 ml plastic flasks (75 cm^2) at 10^6 cells per flask. Twenty-four hours past seeding, UCl-B cells were infected with 10^4 focus forming units of MLV and clone A31 cells were infected with 10^4 focus forming units of MSV or 10^4 infective units of MLV. Cells were grown in Eagle's minimal essential media with 10% fetal bovine serum and fluid changed on the 4th day post seeding.

The RDP was extracted as proposed by Ross, et al.[11] except that 0.1% Triton DN-65 was used to solubilize the RDP activity in solutions

containing ~0.5 mg/ml protein. MLV virions were obtained from the growth medium of MLV-infected UCl-B cells and partially purified by differential centrifugation. The RDP activity was solubilized with 0.1% Triton DN-65.

The first four activity sources were obtained in one experiment. The growing UCl-B cell extract (mitotic index = 5%) was obtained in a separate experiment. MLV virion extract, MLV-transformed UCl-B cell extract and MLV-infected clone A31 cell extract assays contained 0.04 µg 0.46 µg and 1.9 µg protein, respectively, when using a poly-rA template and Mn^{2+}; and 0.4 µg, 4.6 µg and 3.9 µg protein, respectively, in the remaining assays. MSV-transformed clone A31 cell extract assays and growing UCl-B cell extract assays contained 4.9 µg and 4.1 µg protein, respectively. E.coli DNA polymerase I assays contained 1.2×10^{-2} µg protein when using poly-rA templates with Mg^{2+} and 1.2×10^{-3} µg protein in the remaining assays.

Table II

E. coli DNA Polymerase I Activity

	poly-rA:oligo-dT 0.5 µg:0.5 µg	poly-dA:oligo-dT 0.5 µg:0.5 µg	ratio
assay conditions B	10.9*	19.9*	0.55**
assay conditions A minus 100 mM KCl minus 0.01% Triton DN-65	10.3	17.2	0.60
assay conditions A minus 0.01% Triton DN-65	12.7	21.1	0.60
assay conditions A minus 100 mM KCl	36.3	43.6	0.83
assay conditions A	181.3	173.6	1.05

* Numbers given are activity in pmole/hr per µg protein.

** Numbers are obtained by dividing the activity in column 1 by the activity in column 2.

Assay conditions are as given in Table I and contained 0.1 mM Mn^{2+} and 1.2×10^{-3} µg protein.

Table III

Detection of DNA-Dependent DNA Polymerase Activity in Mixed Assays with A Polymerase Extract of MLV -Transformed UCl-B Cells

MLV -transformed UCl-B cell extract (protein in assay)	E. coli DNA polymerase I (protein in assay)	poly-rA:oligo-dT 0.5 µg:0.5 µg	poly-dA:oligo-dT 0.5 µg:0.5 µg
0.46 µg	--	67.2*	0.3
-	0.12 ng	10.1	15.3
-	0.024 ng	2.0	2.8
0.46 µg	0.12 ng	69.5	15.1
0.46 µg	0.024 ng	56.1	3.0

* Numbers given are activity in pmole/hr per assay.

Assays were done in duplicate as described in Table I. for a Mn^{2+} containing condition A assay, except that the glycerol concentration was 4%.

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