Nucleic Acids Research, 1994, Vol. 22, No. 24 5341–5346

Sarkosyl block of transcription reinitiation by RNA polymerase II as visualized by the colliding polymerases reinitiation assay

Marilyn N.Szentirmay and Michèle Sawadogo*
Department of Molecular Genetics, The University of Texas, M.D.Anderson Cancer Center, Houston, TX 77030, USA

Received August 19, 1994; Revised and Accepted November 2, 1994

ABSTRACT

There are indications that different concentrations of Sarkosyl can block transcription initiation by RNA polymerase II *in vitro* at different functional steps [Hawley and Roeder (1985) *J. Biol. Chem.* 260, 8163–8172]. Consequently, this reagent could be a very useful tool for mechanistic studies. So far, however, evidence for the selectivity of Sarkosyl effects on RNA polymerase II transcription has been only indirect. To directly investigate the effect of Sarkosyl on transcription initiation and reinitiation by RNA polymerase II, we employed the reinitiation assay based on utilization of templates containing G-free cassettes (colliding polymerases reinitiation assay, or CoPRA). These experiments showed unambiguously that, under the appropriate conditions, Sarkosyl can be used to block transcription reinitiation by RNA polymerase II while allowing a first round of initiations from preassembled initiation complexes. This inhibition is not due to a disruption of the SII-dependent elongation of the reinitiated transcripts, and the levels of Sarkosyl that prevent transcription reinitiation coincide with the levels that block preinitiation complex assembly. However, Sarkosyl addition to transcription reactions reconstituted with partially purified transcription factors was found to have several undesirable side effects. The usefulness and limitations of the Sarkosyl-based and CoPRA assays for measurements of transcription reinitiation are discussed.

INTRODUCTION

Biochemical analyses of the basic mechanisms by which RNA polymerase II (RNAPII) initiates transcription are providing insights that are pivotal in understanding the transcriptional control of eukaryotic genes. Transcription initiation has been resolved into a number of steps (reviewed in 1–3) that involve not only the multisubunit enzyme RNAPII, but also general factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH) and gene-specific regulatory proteins (4). Initial activation of a gene can be thought of as the assembly at the promoter of a preinitiation complex, which will initiate the synthesis of a first transcript. Sustained transcription of a gene necessitates reinitiation, a process that may be controlled independently from the initial activation (5).

Any study of transcription reinitiation must rely on a means to distinguish first-round transcription initiation from subsequent initiations at the same promoter. In cases where more than one transcript per gene are produced, simple quantitation can reveal that reinitiation is occurring (6). Commonly, however, RNAPII transcription *in vitro* is inefficient, yielding less than one transcript per gene. A different approach is therefore required to explore reinitiation. In addition, even in experiments where investigating reinitiation is not a primary goal, it is often desirable to limit transcription to a single round by blocking reinitiation. Agents that inhibit initiation but not elongation by the RNA polymerase can be used in both of these cases. For example, transcription initiation but not elongation by *Escherichia coli* RNA polymerase is sensitive to inhibition by the antibiotic rifampicin (7), which is routinely used to limit prokaryotic transcription to a single round. Similarly, the anionic detergent Sarkosyl has been used to block initiation steps by the eukaryotic RNA polymerases *in vitro* (8–19), and a number of determinations of RNAPII reinitiation have relied on the comparison of transcription levels in the presence and absence of Sarkosyl (11–15). At best, however, the use of Sarkosyl to study reinitiation is indirect and complex. This detergent affects various steps of transcription initiation and elongation, and valid interpretation of Sarkosyl blocking experiments requires careful analysis and extensive control experiments (11–13). Furthermore, the specificity of Sarkosyl was originally established in extracts and crude reconstituted systems (8–13); its performance in more purified systems has not been well characterized.

An alternative and more direct assay for transcription reinitiation by RNAPII is based on electrophoretic separation of transcripts produced from templates containing a G-free cassette (20). With such templates and in the absence of GTP, the RNAPII

*To whom correspondence should be addressed

molecules responsible for the first round of transcription remain at the end of the G-free region, creating a block to complete elongation of the reinitiated transcripts. As a result, the successive polymerases stacked at the end of the cassette produce transcripts whose lengths decrease stepwise with each round of initiation (21–23). This assay, which for brevity we call CoPRA (colliding polymerases reinitiation assay) therefore permits direct visualization of transcripts resulting from successive rounds of initiation at a promoter.

We employed CoPRA with partially purified transcription factors to assess the effect of Sarkosyl on reinitiation by RNAPII. These experiments demonstrated unambiguously that, if conditions are properly chosen, Sarkosyl can block transcription reinitiation by RNAPII while allowing a single round of transcription by preassembled initiation complexes. However, we found that, apart from its direct effect on transcription, Sarkosyl can also indirectly alter the yield of *in vitro* transcripts. Comparison of the CoPRA and Sarkosyl-based measurements of reinitiation revealed the usefulness and limitations of both assays.

MATERIALS AND METHODS

Reagents

A 7.5% stock solution (pH 8.0) of the sodium salt of Sarkosyl was prepared by addition of NaOH to N-lauroylsarcosine (Sigma). Ribonucleoside triphosphates were purified by chromatography on Polyanion SI (Pharmacia).

Purification of transcription factors and RNA polymerase II

Recombinant proteins TFIIB and SII were expressed in *E.coli* and purified as previously described (23). The recombinant proteins were diluted in BC100 (20 mM Tris pH 7.3, 0.2 mM EDTA, 20% glycerol, 100 mM KCl) containing 1 mM dithiothreitol and 0.1% Triton-X-100. HeLa transcription factors TFIID and TFIIE/F/H, HeLa phosphocellulose (Whatman P11) fractions, and human RNA polymerase II were prepared by published procedures (20,24). Factors were stored at −80°C in BC100 with 10 mM 2-mercaptoethanol or 1 mM dithiothreitol.

In vitro transcription

Transcription reaction mixtures (25 μL) contained 60 mM KCl, 10–15 mM $(NH_4)_2SO_4$, 12 mM Tris (pH 7.3 at 25°C), 12% glycerol, 3.6% (wt/vol) polyethylene glycol 8000, 40 mM HEPES (pH 8.4), 7.5 mM $MgCl_2$, 0.12 mM EDTA, 4 mM dithiothreitol, 0.6 mM ATP and UTP, 25 μM [^{32}P]CTP (5000–10000 cpm/mmole), 8 units RNasin (recombinant ribonuclease inhibitor, Promega), and 400 ng of template pML(C_2AT)19Δ-50. Unless otherwise noted in the Figure legends, reaction mixtures contained recombinant TFIIB (1.3–3 U), TFIIE/F/H (Biogel A–1.5m fraction, 1.0 U), RNAPII (1.3 U), TFIID (DE52 fraction, 0.3 U), and recombinant SII (15 ng). Reaction mixtures were incubated at 30°C according to protocols described in each figure legend.

Preincubation of factors with the template were carried out in 20 μL reaction volumes of the above composition , except they contained 4.5% (wt/vol) polyethylene glycol 8000 and 5 mM dithiothreitol, and lacked nucleotides. Sarkosyl and nucleotides were then added to bring the total reaction volume to 25 μL and the transcription composition shown above. Reactions were stopped and transcripts prepared for electrophoresis through 4.5% polyacrylamide gels as previously described (25). Transcription gels were quantitated with a betascanner (Betascope 600, Betagen).

RESULTS

Effect of Sarkosyl on the transcription of G-free cassette templates

Experiments by Hawley and Roeder have demonstrated that transcription initiation by RNAPII can be resolved into several steps that differ in sensitivity to inhibition by Sarkosyl (11,12). These investigators observed that low levels of Sarkosyl blocked assembly of a transcription preinitiation complex, or 'rapid start complex', which will rapidly initiate transcription upon addition of nucleotides. The threshold Sarkosyl concentration required to prevent preinitiation complex assembly depended on the source of transcription factors, with cruder systems demanding higher

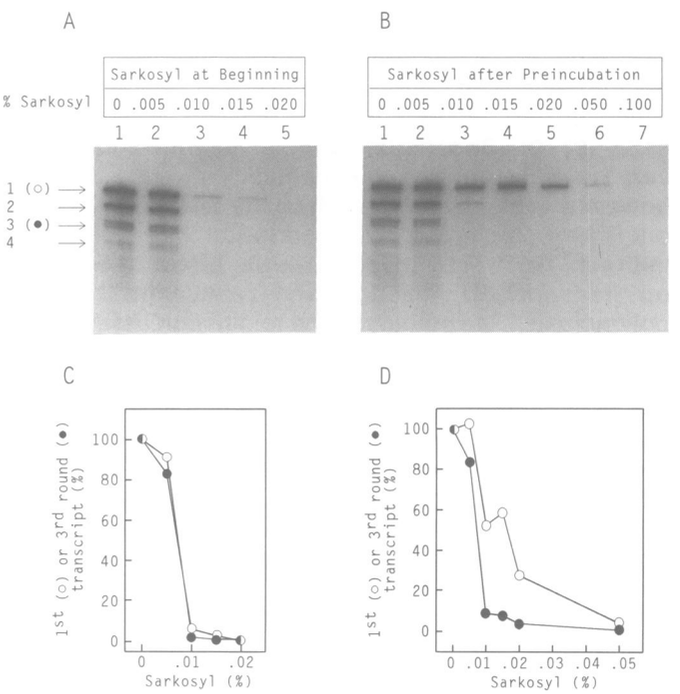

Figure 1. Effect of Sarkosyl on transcription initiation and reinitiation by RNAPII. Sarkosyl titrations were carried out using CoPRA and a reconstituted system composed of a mixture of recombinant proteins and semi-purified HeLa transcription factors and RNAPII as described in the Materials and Methods. (**A**) Effect of Sarkosyl when added at the beginning of the transcription reaction. Various concentrations of Sarkosyl (% wt/vol), as indicated above each lane, were included from the beginning of a 70 min transcription reaction. Migration in the gel of the full-length transcripts resulting from the first round of initiation (1) and migration of the second-, third- and fourth-round transcripts (2,3,4) are indicated at left. (**B**) Effect of Sarkosyl on preformed preinitiation complexes. After a 40 min preincubation of RNAPII, factors and DNA, as described in the Materials and Methods section, Sarkosyl and nucleotides were added and transcription was carried out for 60 min. Sarkosyl concentrations in the 25 μL final reaction volume are indicated above each lane. (**C**) Quantitation of the experiment shown in panel A. The amount of first-round transcripts (open circles) and, as a measure of reinitiation, the amount of third-round transcripts (filled circles) were determined by betascanning and plotted for the various Sarkosyl concentrations as the percentages of the amounts present in the absence of inhibitor. (**D**) Plot of the quantitative data derived from the experiment shown in panel B.

levels of Sarkosyl (0.015% Sarkosyl for reactions reconstituted from HeLa nuclear extract-derived phosphocellulose fractions, versus 0.025% for the unfractionated extract). The extent of reinitiation was measured after completion of preinitiation complex formation by comparing transcription levels in the presence and absence of Sarkosyl.

In the CoPRA analysis, the presence or absence of a pattern of decreasing size transcripts provides a direct indication of the extent of reinitiation (22). We wished to confirm with this straightforward assay whether Sarkosyl can indeed limit transcription to a single round and to evaluate the use of this inhibitor in reactions reconstituted from more purified transcription factors. For these analyses, we used a template containing the adenovirus major late promoter driving transcription of the G-free cassette (pML(C_2AT)19Δ-50, ref. 21). Sarkosyl titrations were performed in reconstituted transcription reactions containing recombinant TFIIB and SII and partially purified TFIID, TFIIE/F/H, and RNAPII (23). In the absence of Sarkosyl, the expected pattern of decreasing-length transcripts resulting from multiple rounds of initiation by RNAPII was observed (Fig. 1A and B, lanes 1). When Sarkosyl was added at the beginning of the reaction (i.e. prior to any protein-DNA interaction), a concentration of 0.005% allowed first-round and subsequent initiations, but concentrations of 0.01−0.015% or greater prevented nearly all transcription (Fig. 1A); in the latter case there was no quantitative difference in the Sarkosyl sensitivity of first-round versus reinitiated transcripts (Fig. 1C). These Sarkosyl concentrations coincide with those shown to prevent preinitiation complex assembly in reactions reconstituted with phosphocellulose fractions (11).

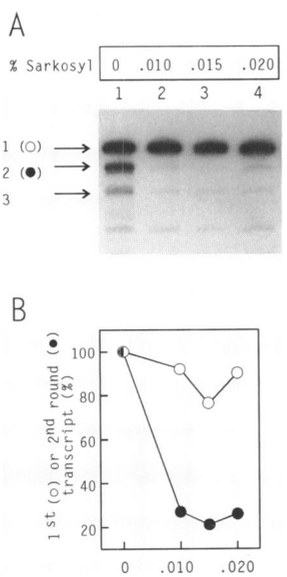

Figure 2. Selective block of transcription reinitiation by Sarkosyl. Conditions were similar to those shown in Fig. 1B, except that preincubation of transcription factors, RNAPII and template DNA was for 25 min and the duration of transcription after addition of Sarkosyl and nucleotides was very short (7 min). (**A**) Autoradiogram of the CoPRA gel. Concentrations of Sarkosyl (% wt/vol) in each reaction are indicated on the top. (**B**) Plot of the quantitative results derived from the experiment shown in panel A, with the amount of first-round (open circles) and second-round transcripts (filled circles) expressed as percentages of the amounts present in the absence of Sarkosyl.

When increasing concentrations of Sarkosyl were added after an initial incubation of the DNA and protein factors and immediately prior to the addition of nucleotides, reinitiation was preferentially affected (Fig. 1B). At 0.015% Sarkosyl, there were virtually no reinitiated transcripts, but the number of first-round transcripts also decreased (Fig. 1B, compare lane 4 to lanes 1 and 2. See also the quantitation in Fig. 1D). In agreement with the earlier observations, Sarkosyl concentrations greater than 0.02% significantly inhibited first-round transcription, even after the factors and the DNA had been preincubated. Concentrations of 0.05% and greater eliminated nearly all transcription, presumably by attacking the preformed preinitiation complexes or interfering with the transcription initiation process itself (Fig. 1B, lanes 6 and 7).

Using Sarkosyl to constrain transcription to a single round

From the results of the Sarkosyl titration shown in Fig. 1B, it seemed that Sarkosyl levels that totally blocked reinitiation could also, though to a lesser extent, interfere with the ability of preassembled complexes to initiate and complete the first round of transcription. We attempted to find conditions under which Sarkosyl would more selectively block reinitiation without affecting the production of first-round transcripts. When the incubation time after addition of Sarkosyl and nucleotides was limited to only a few minutes, Sarkosyl concentrations of 0.01% or greater blocked reinitiation with very little decrease in the yield of first-round transcripts (Fig. 2A, lanes 2−4). Furthermore, the threshold level of Sarkosyl (0.010−0.15%) required under these conditions to prevent transcription reinitiation corresponded precisely to the level that blocked preinitiation complex assembly (Fig. 1A). Note that during this short transcription time the reaction without Sarkosyl produced only a limited number of transcripts from reinitiation (Fig. 2A, lane 1). Nevertheless, it is clear that under these conditions, Sarkosyl in the range 0.01−0.02% selectively prevented reinitiation without

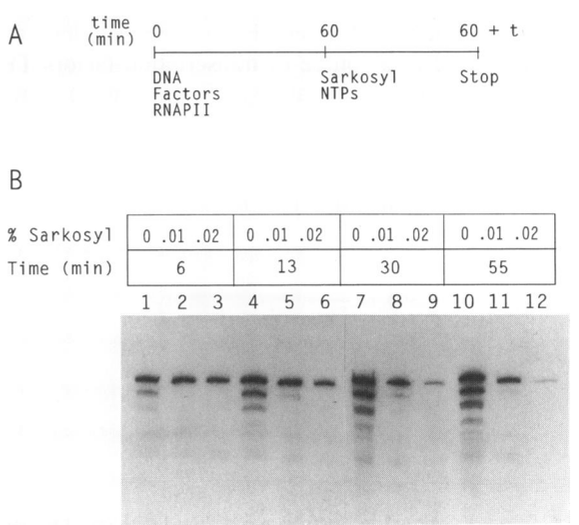

Figure 3. Time course of transcription after Sarkosyl addition. (**A**) Diagram of the experimental protocol, where various transcription times (t) were used after addition of Sarkosyl to preincubated mixtures of transcription factors and DNA. (**B**) Autoradiogram of the CoPRA gel. Sarkosyl and transcription times are indicated above each lane.

significantly affecting first-round initiation by pre-assembled transcription complexes (Fig. 2B).

Ancillary effects of Sarkosyl

The experiments with short transcription times proved useful in visualizing the block of reinitiation by Sarkosyl and allowed determination of the range of Sarkosyl concentrations (0.01−0.02%) that affected reinitiation selectively. This same range was used to gauge the effect of Sarkosyl as a function of transcription time (Fig. 3). Factors and template DNA were preincubated for 60 min to allow formation of preinitiation complexes. Different concentrations of Sarkosyl were then added, immediately prior to nucleotide addition, and the transcription reactions were allowed to proceed for various periods of time (Fig. 3A). In the absence of Sarkosyl, first-round transcripts accumulated rapidly, and the amount of reinitiated transcripts, as well as the number of rounds of reinitiation, increased progressively with time (Fig. 3B, lanes 1, 4, 7, and 10). In the presence of Sarkosyl, first-round transcription from preformed preinitiated complexes was expected to level off rapidly. However, instead of a constant level of first-round transcripts at longer times and increasing Sarkosyl levels, first-round transcripts actually decreased in a time- and Sarkosyl-dependent fashion (Fig. 3). This result clearly indicates that transcript degradation can be induced, or at least greatly enhanced, by the addition of Sarkosyl. This Sarkosyl-dependent decay of transcripts was only observed when Sarkosyl was present during RNA synthesis, but not when Sarkosyl was added after transcription had been halted by α-amanitin (data not shown). Our interpretation of these results is that Sarkosyl can prevent proteins from interacting with the nascent transcripts, which increases the accessibility of these RNAs to nucleases. However, one could also invoke other mechanisms such as a dissociation by Sarkosyl of endogenous RNase-RNase inhibitor complexes.

An additional effect of Sarkosyl was revealed when we attempted to use this inhibitor in a CoPRA assay performed with cruder preparations of the transcription factors. As noted previously (22), transcripts corresponding to several rounds of initiation were observed when the phosphocellulose 0.5 M fraction was used as a source of transcription factors TFII-B, E, F and H in the absence of Sarkosyl (Fig. 4, lane 1). However, addition of even very low levels of Sarkosyl (0.005%) resulted in the appearance of heterogeneous transcripts longer than the G-free cassette (Fig. 4, lanes 2 and 3), indicating that GTP was released from some components in the fraction. Under these conditions, the polymerases were no longer arrested at a single point on the template, and the characteristic pattern of first-round and reinitiated transcripts disappeared (lane 3). This is similar to the case of transcription in nuclear extracts, where CoPRA cannot be used because the levels of endogenous GTP are sufficient to allow extension of transcripts past the end of the G-free cassette.

SII-dependent extension of reinitiated transcripts occurs at Sarkosyl levels that block reinitiation

Sarkosyl is known to affect elongation by RNAPII, in some cases increasing elongation efficiency (26,27) and in others inhibiting elongation (11, 28−31). Increased pausing by RNAPII can occur via inhibition of the elongation factor SII at sufficiently high Sarkosyl concentrations (29,30). Since SII is required for elongation of reinitiated but not first-round transcripts (23), apparent inhibition of reinitiation by Sarkosyl could occur by interference with SII-dependent elongation. To test this possibility, transcription reactions were carried out in the absence of SII, producing full-length first-round transcripts as well as incompletely elongated reinitiated transcripts (Fig. 5B, lane 1; see ref. 23). After this initial period of transcription, Sarkosyl and SII were added to determine whether the reinitiated transcripts could undergo SII-dependent elongation at Sarkosyl levels that block reinitiation (Fig. 5A). SII-assisted elongation was observed at 0.02% Sarkosyl as well as without Sarkosyl, resulting in appearance of the characteristic pattern of fully elongated reinitiated transcripts (Fig. 5B, lanes 4 and 5). This result clearly

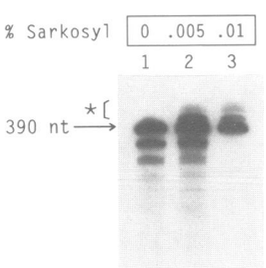

Figure 4. Effect of Sarkosyl in reactions reconstituted with crude preparations of transcription factors. Transcription factors, in which SII and TFII-B, E, F and H were supplied by the phosphocellulose 0.5 M step fraction from HeLa nuclear extract, were preincubated with the template DNA for 25 min prior to the addition of Sarkosyl and nucleotides. Transcription reactions were carried out for 20 min. Migration of the 390-nucleotide full-length G-free transcript is indicated by the arrow. Longer transcripts, due to the release of GTP, are indicated by the asterisk.

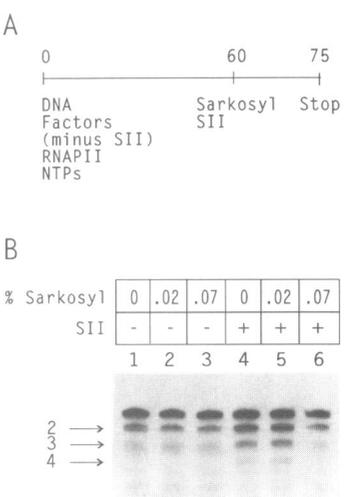

Figure 5. SII-dependent elongation of reinitiated transcripts occurs at Sarkosyl levels that block reinitiation. (**A**) Diagram of the experimental protocol. Transcription reactions were carried out in the absence of SII for an initial 60 min period. Recombinant SII (15 ng) and various concentrations of Sarkosyl were then added, and the reactions were allowed to continue for another 15 min. (**B**) Autoradiogram of the CoPRA gel. The concentrations of Sarkosyl present during the final incubation period are indicated above each lane. Migration of the fully elongated second-, third- and fourth-round transcripts that accumulate in the presence of active SII is indicated at left.

demonstrates that Sarkosyl does not affect SII-dependent RNAPII elongation within this concentration range. In fact, a much higher concentration of Sarkosyl (0.07%) was required to prevent SII-dependent elongation of the reinitiated transcripts (Fig. 5B, lane 6), in agreement with the previously reported levels of Sarkosyl (>0.05%) that inhibit elongation by the SII-RNAPII complex (30). Consequently, the specific inhibition of reinitiation observed at 0.01–0.02% Sarkosyl is clearly not caused by an inhibition of SII-dependent transcript elongation. Instead, as originally postulated by Hawley and Roeder, it is probably the reassembly of preinitiation complexes that is affected (11–13).

DISCUSSION

The application of Sarkosyl to block transcription reinitiation by RNAPII depends on the selectivity of Sarkosyl action. We used an independent assay of reinitiation (CoPRA) to gauge the effects of Sarkosyl and found the two assays consistent with one another. Previously, the inference that Sarkosyl can block reinitiation was based on convincing, but indirect, analysis. The CoPRA assay showed unambiguously that, under appropriate conditions, Sarkosyl can indeed block reinitiation preferentially while allowing a first round of initiation from preassembled initiation complexes. This direct demonstration is very important because there are basically no other reinitiation-blocking agents that can be used for mechanistic studies with the RNA polymerase II system.

The levels of Sarkosyl that affect various transcription steps could be determined only after adjusting the reaction conditions to minimize several side effects of this inhibitor. We found that, apart from its effects on transcription initiation and elongation, Sarkosyl could markedly alter the production of transcripts in at least two different ways, depending on experimental conditions. First, Sarkosyl could release GTP (and therefore probably also other tightly bound cofactors) from components present in some chromatographic fractions. Second, Sarkosyl could promote nascent transcript degradation, presumably by preventing binding of proteins that normally protect these RNAs against nuclease attack. Consequently, the utility of Sarkosyl as a reinitiation-blocking agent depends critically on the precise determination of concentration ranges and conditions under which transcription can be limited to a single round while minimizing all of the Sarkosyl side effects.

Because Sarkosyl is after all a detergent, it is not surprising to find that it can affect interactions among proteins, RNA, and nucleotides other than those targeted. In fact, a drawback to the use of Sarkosyl to block reinitiation is that it is quite difficult to predict and control for all the conceivable consequences of Sarkosyl addition. For example, since Sarkosyl may dissociate complexes, it could alter the critical balance between transcriptional inhibitors or activators present in semi-purified fractions. We found that the acceleration of transcript breakdown by Sarkosyl is a concentration-dependent phenomenon, so using the lowest possible concentrations of Sarkosyl to block transcription would limit this undesirable effect. In some instances, the CoPRA assay may be useful in optimizing the amount of Sarkosyl required to block reinitiation or evaluating other putative reinitiation-blocking agents. Clearly, the two methods are complementary for some uses, with CoPRA better suited with purified or partially purified fractions (where GTP is likely to be absent), and Sarkosyl experiments more appropriate in cruder fractions where endogenous GTP levels are high enough to preclude the use of G-free cassette templates. In addition to permitting direct visualization of reinitiation, CoPRA reveals the number of rounds of productive initiation per transcribed gene and the distribution of transcripts among these different rounds.

The levels of Sarkosyl required to block reinitiation in our system reconstituted with a mixture of partially purified initiation factors and recombinant proteins correspond exactly to the levels found earlier to block reinitiation with phosphocellulose fractions. We showed that these same levels of Sarkosyl do not inhibit the activity of SII (Fig. 5), which is an important result in light of the fact that SII is required for full extension of the reinitiated transcripts (23). Since these Sarkosyl levels that block reinitiation but not SII activity were found, both here and in earlier studies, to also prevent assembly of first-round preinitiation complexes, our observations are consistent with the model originated by Hawley and Roeder, in which each round of initiation requires reassembly of a preinitiation complex (11–13). However, this simple model does not preclude possible differences between the composition or assembly of first-round and subsequent complexes.

ACKNOWLEDGEMENTS

We thank S.Maity and M.W.Van Dyke for critical review of the manuscript. This work was supported in part by Grant GM38212 from the National Institutes of Health.

REFERENCES

1. Buratowski,S. (1994) *Cell*, **77**, 1–3.
2. Zawel, L. and Reinberg,D. (1993) *Progr. Nucl. Acids Res.* **44**, 67–108.
3. Conaway,J. and Conaway,R. (1991) *J. Biol. Chem.*, **266**, 17721–17724.
4. Tjian,R. and Maniatis,T. (1994) *Cell*, **77**, 5–8.
5. Jiang,Y. and Gralla,J.D. (1993) *Mol. Cell. Biol.*, **13**, 4572–4577.
6. Bogenhagen,D.F., Wormington,W.M. and Brown,D.D. (1982) *Cell*, **28**, 413–421.
7. Sippel,A. and Hartmann,G. (1968) *Biochim. Biophys. Acta*, **157**, 218–219.
8. Gariglio,P., Buss,J. and Green,M.H. (1974) *FEBS Lett.*, **44**, 330–333.
9. Ackerman,S., Bunick,D. Zandomeni,R. and Weinmann,R. (1983) *Nucleic Acids Res.*, **11**, 6041–6064.
10. Tolunay,H.E., Yang,L., Anderson,W.F. and Safer,B. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 5916–5920.
11. Hawley,D.K. and Roeder,R.G. (1985) *J. Biol. Chem.*, **260**, 8163–8172.
12. Hawley,D.K. and Roeder,R.G. (1987) *J. Biol. Chem.*, **262**, 3452–3461.
13. Kadonoga,J.T. (1990) *J. Biol. Chem.* **265**, 2624–2631.
14. Carcamo,J., Lobos,S., Merino,A., Buckbinder,L., Weinmann,R., Natarajan,V. and Reinberg,D. (1989) *J. Biol. Chem.*, **264**, 7704–7714.
15. Arnosti,D.N., Merino,A., Reinberg,D. and Schaffner,W. (1993) *EMBO J.*, **12**, 157–166.
16. White,J., Brou,C., Wu,J., Lutz,Y., Montcollin,V. and Chambon, P. (1992) *EMBO J.*, **11**, 2229–2240.
17. Kovelman,R. and Roeder,R.G. (1990) *Genes Dev.*, **4**, 646–658.
18. Kunkel,G.R. and Danzeizer,D.A., (1992) *J. Biol. Chem.*, **267**, 14250–14258.
19. Schultz,M.C., Choe,S.Y., and Reeder,R.H. (1993) *Mol. Cell. Biol.*, **13**, 2644–2654.
20. Sawadogo,M. and Roeder,R.G. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4394–4398.
21. Sawadogo,M. and Roeder,R.G. (1985) *Cell*, **43**, 165–175.
22. Szentirmay,M. and Sawadogo,M. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 10691–10695.
23. Szentirmay,M. and Sawadogo,M. (1993) *EMBO J.*, **12**, 4677–4684.
24. Van Dyke,M.W., Roeder,R.G. and Sawadogo,M. (1988) *Science*, **241**, 1335–1338.

25. Dignam,J.D., Lebowitz,R.M. and Roeder,R.G. (1983) *Nucleic Acids Res.*, **11**, 1475–1489.
26. Laspia,M.F., Wendel,P. and Mathews,M.B. (1993) *J. Mol. Biol.*, **232**, 732–746.
27. Izban,M.G. and Luse,D.S. (1991) *Genes Dev.* 5, 683–696.
28. Kash,S.F., Innis,J.W., Jackson,A.U. and Kellems,R.E. *(1993) Mol. Cell. Biol.*, 13, 2718–2729.
29. Reines,D., Chamberlin,M.J. and Kane,C.M. (1989) *J. Biol. Chem.*, **264**, 10799–10809.
30. Wiest,D.K., Wang,D., and Hawley,D.K. (1992) *J. Biol. Chem.*, **267**, 7733–7744.
31. Wiest,D.K, and Hawley,D.K. (1990) *Mol. Cell. Biol.*, **10**, 5782–5795.