these domains. One can imagine for example that the initial angle between the faults in the EM and the CM was close to the optimal failure direction for N5°E compression three million years ago; the fault azimuths at that time would have been N35°E and N25°W, respectively. For the assumption that the maximum stress bisects the angle between these sets also today, with the EM faults oriented N85°E and CM ones at N45°W, the present day compression direction should be N20°E. This direction would then imply a 15° clockwise rotation of the stress, a 55° clockwise rotation of the EM blocks, and a 20° counterclockwise rotation of the CM blocks.
12. The discrepancy between the surface-mapped east-west Manix and the ~N20°W direction of the 1947 Manix rupture was first pointed out by C. F. Richter and J. M. Nordquist [*Seismol. Soc. Am. Bull.* **41**, 347 (1951)] and detailed by C. F. Richter [*Elementary Seismology* (Freeman, San Francisco, CA, 1968)]. D. Doser has carefully reanalyzed this event and its aftershocks [*Seismol. Soc. Am. Bull.* **80**, 267 (1990)].
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18. Supported in the past by the National Aeronautics and Space Administration's geodynamic program, by past and current grants from the Israel-U.S. Binational Science Foundation, and by a National Science Foundation Presidential Young Investigator Award (G.C.B.). We thank G. Mavko and J. Dvorkin for their helpful discussions and W. Thatcher and P. Bird for insightful comments.

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Interaction Between Transcription Regulatory Regions of Prolactin Chromatin

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The regulation of transcription requires complex interactions between proteins bound to DNA sequences that are often separated by hundreds of base pairs. As demonstrated by a nuclear ligation assay, the distal enhancer and the proximal promoter regions of the rat *prolactin* gene were found to be juxtaposed. By acting through its receptor bound to the distal enhancer, estrogen stimulated the interaction between the distal and proximal regulatory regions two- to threefold compared to control values. Thus, the chromatin structure of the *prolactin* gene may facilitate the occurrence of protein-protein interactions between transcription factors bound to widely separated regulatory elements.

Three models have been proposed to explain the mechanism by which transacting factors act at a distance: the scanning model, the structural transmission model, and the DNA looping model (*1*). Data have been obtained in support of the DNA looping model, in which the intervening DNA sequence between the DNA-bound transacting factor and the transcription initiation complex is looped out, but it is unclear what drives the formation of the loops (*2*). Interacting proteins may extend and contact one another, bridging the distance between the proteins and forcing the intervening DNA to loop out (*3*). Alternatively, the DNA may be intrinsically bent, thereby allowing widely separated regions of DNA to be juxtaposed. Over short distances [200 to 300 base pairs (bp)], supercoiling may provide enough bending of the DNA to allow association between DNA binding proteins (*4*). Over large distances, the packaging of the DNA into chromatin may orient two widely separated protein binding sites to permit the association of the DNA binding proteins (*5*).

Expression of the rat *prolactin* (*PRL*) gene is regulated by a number of different polypeptide and steroid hormones that act through two distinct regulatory regions separated by approximately 1500 bp (Fig. 1) (*6*). The steroid hormone estrogen (E2) induces the transcription of the *PRL* gene by binding to the estrogen receptor (ER), which in turn binds to the estrogen response element (ERE) (*7*). This element is located at the 3′ end of the distal enhancer region between −1550 and −1578 bp (*8*). How the ER complex influences the activity of RNA polymerase II located 1500 bp downstream at the promoter is unknown. Data from studies with a cell-free transcription system containing purified ER and templates with an ERE a short distance from the promoter led Elliston *et al.* (*9*) to suggest that the ER enhances transcription by facilitating the formation of a stable preinitiation complex. For the ER complex to perform a similar function in the *PRL* gene in vivo, the spatial distance between the ER complex and the promoter must be reduced. We have previously shown that the chromatin surrounding the ERE and the promoter becomes hypersensitive to nucleases after treatment of cells with E2, although the region between the ERE and the promoter remains insensitive to nucleases (*10*). This suggests that the ER complex does not scan along the DNA to the promoter nor does the activated ER complex initiate a change in the DNA structure that is propagated from the ERE to the promoter.

To determine if DNA looping may facilitate the interaction between the ER and the transcription initiation complex, we examined the chromatin looping potential of the 5′ upstream regulatory elements of *PRL* (Fig. 2A) (*11*) with a modified DNA looping assay of Mukherjee *et al.* (*12*). We used *PRL*-Tn*5*–bovine papillomavirus (BPV) minichromosomes as our source of *PRL* chromatin. These minichromosomes are packaged into nucleosomal arrays (*10*) and replicate extrachromosomally at a level of 40 to 60 copies per cell in a stable, clonal cell line (G1I) obtained by the transfection of rat pituitary GH_3 cells with the *PRL*-Tn*5*-BPV vector. The transcription of the Tn*5* gene, which acts both as a selectable marker (G418 resistance) and as a reporter, is under the control of the *PRL* regulatory elements (Fig. 1) and can be induced by E2 (*13*). When nuclei isolated from G1I cells were partially digested with Pst I, a number of different *PRL*-Tn*5* chromatin fragments were produced that could theoretically form Pst I ligation products (Fig. 2B). Polymerase chain reaction (PCR)–mediated analysis principally detected only the formation of Pst I ligation products that correspond to the P_3-P_4 and P_3-P_5 *PRL* chromatin loops (Fig. 2C, lane 5).

The PCR products were analyzed by

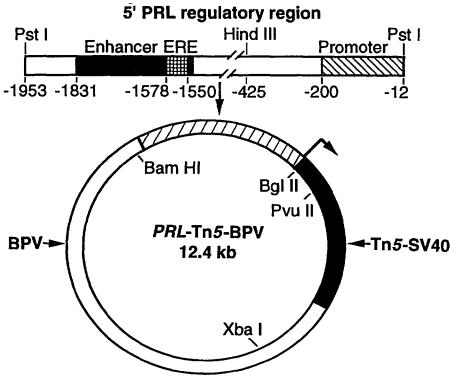

Fig. 1. The *PRL*-Tn*5*-BPV minichromosome. The minichromosome contains the transcription regulatory elements (hatched) of the rat *PRL* gene (−1953 to −12), which controls the expression of the Tn*5*-SV40 reporter gene (solid). The entire BPV genome is present in the minichromosome (unshaded). Further details on the characterization of the cell lines containing the *PRL*-Tn*5*-BPV minichromosome have been described (*10, 13*).

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restriction endonuclease mapping and dideoxynucleotide sequencing and were shown to be the result of the predicted Pst I chromatin fragment ligations (*14*). If these were corrected for the amount of Pst I chromatin fragments available for ligation (*15*), similar amounts of P_3-P_4 and P_3-P_5 *PRL* chromatin loops were formed (*16*). By comparing signal intensities of the 871-bp P_3-P_4 PCR product with the 702-bp PCR internal standard (*17*), we estimate that 30 to 50% of the P_3-P_4 *PRL* chromatin fragments formed were ligated. The formation of chromatin loops between the distal enhancer and proximal promoter could also be detected with restriction endonucleases other than Pst I. Digestion of isolated nuclei with Bam HI, which cuts 286 bp 5′ of the P_3 site, and Bgl II, which cuts 14 bp 3′ of the P_4 site (Fig. 1), followed by treatment with T4 DNA ligase and PCR-mediated analysis resulted in the production of a single PCR fragment of 1071 bp (*18*).

The formation of these Pst I ligation products was dependent on treatment of isolated nuclei with both Pst I and T4 DNA ligase (Fig. 2C). These ligation products were not formed as a result of random ligation of Pst I chromatin fragments. Scanning densitometry revealed that the amount of P_3-P_5 ligation products is approximately eightfold greater than the amount of P_2-P_4 ligation products, even though similar amounts of their corresponding Pst I DNA fragments were produced (Figs. 2B) (*19*). The small amount of detectable ligation product from a P_2-P_4 DNA loop was not entirely the result of inefficient PCR amplification of the longer P_2-P_4 ligation products because in vitro–generated P_2-P_4 ligation products could be PCR-amplified at about 60% of the efficiency of P_3-P_5 ligation products (*18*). In addition, size restrictions on loop formation probably do not play a role because the P_2-P_4 and P_3-P_5 Pst I chromatin fragments differ in length by only 5% and exceed 2000 bp. With purified DNA, Wang and Giaever (*20*) have reported that if two interacting sites are misaligned by 180°, the free energy required to form a loop is independent of the length of DNA if the size of the DNA exceeds 1000 bp.

We examined nuclei isolated from GH_3 cells that do not contain the *PRL* minichromosome to see if loop formation of the endogenous *PRL* chromatin could be detected. PCR amplification of the Pst I ligation products produced fragments of 871 and 973 bp (Fig. 2D). Restriction endonuclease analysis verified that these fragments were produced as a result of ligation of the 5′ distal Pst I site at −1953 bp (P_{1E}) with two Pst I sites located near the proximal promoter at −12 bp (P_{2E}) and +92 bp (P_{3E}), respectively (*14*). The 973-bp PCR product from the endogenous *PRL* gene was observed upon longer exposure of autoradiograms obtained from ligation assays performed on G1I nuclei (*18*). Because PCR products that reflect nuclear ligation in the endogenous gene were observed, we conclude that the specific looped complexes are due to the intrinsic, specific packaging of the *PRL* regulatory region into chromatin structure rather than to an anomaly of the extrachromosomal (episomal) nature of the minichromosome.

We examined the specificity of the Pst I *PRL* chromatin fragment ligations by testing whether chromatin fragments formed by Pst I cleavage in the Tn*5* gene could ligate within G1I nuclei. A number of Pst I chromatin fragments that contain portions of the Tn*5* gene were generated (Fig. 3A). The ligation of the 1154-bp P_4-P_6 Pst I chromatin fragment would generate a 414-bp PCR product, but no 414-bp PCR product was observed in samples obtained from G1I nuclei treated with Pst I and T4 DNA ligase (Fig. 3B, lanes 3 and 4) even though similar amounts of P_4-P_6 and P_3-P_5 Pst I chromatin fragments were produced (compare the intensity of the 2172-bp band in Fig. 2B with that of the 1154-bp band in Fig. 3A relative to the common 3095-bp band seen in both figures). The inability to detect the formation of the P_4-P_6 chromatin loops was not the result of low PCR amplification because the signal intensity of 0.02 fg of the 414-bp PCR standard (*17*) was similar to the signal achieved with the use of 25 fg of the 702-bp standard (compare Fig. 3B, lane 1, with Fig. 2C, lane 2).

To demonstrate that the inability of P_4-P_6 chromatin fragments to ligate is the result of the chromatin structure of this fragment, we purified DNA from G1I nuclei that were treated with Pst I but not with ligase. The DNA was diluted to 0.3 mg/ml (approximately the DNA concentration in the nuclear ligation assay), treated with T4 DNA ligase (either 20 or 100

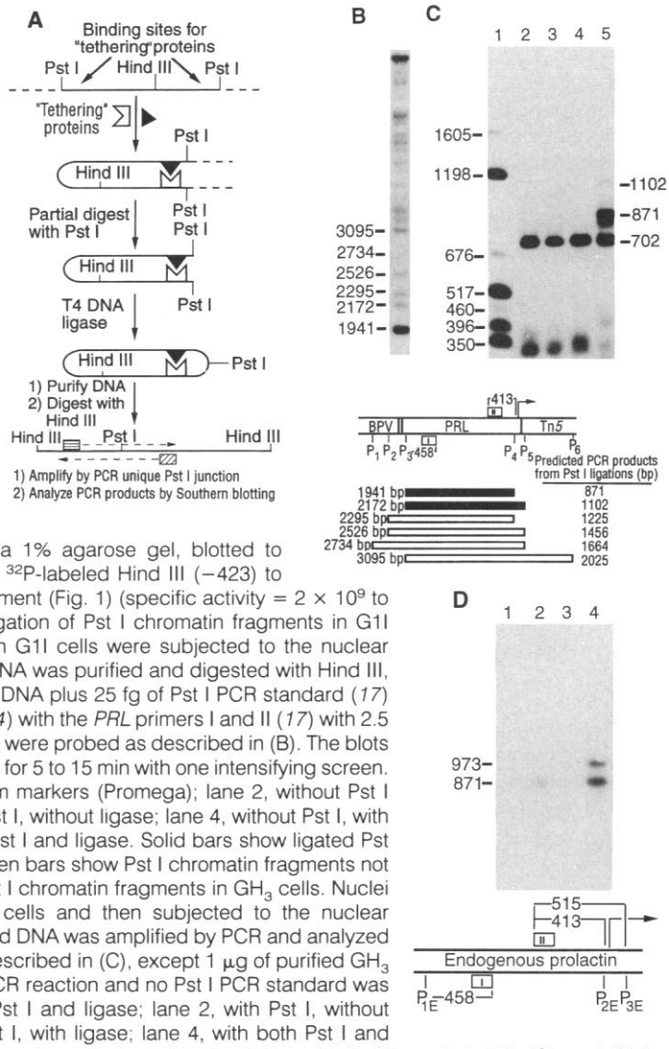

Fig. 2. *PRL* Pst I chromatin fragment ligation. (**A**) Flow chart of nuclear ligation assay (*11*). (**B**) Southern blot of Pst I *PRL*-Tn*5*-BPV DNA fragments. Nuclei were isolated from G1I cells grown in Dulbecco's modified Eagle's media plus 10% fetal bovine serum and then subjected to the nuclear ligation assay (*11*), except an aliquot of Pst I–treated nuclei was removed before treatment with ligase. DNA was purified from this aliquot and digested to completion with Xba I. Ten micrograms of the DNA was separated on a 1% agarose gel, blotted to nylon, and probed with a ^{32}P-labeled Hind III (−423) to Pst I (−12) *PRL* DNA fragment (Fig. 1) (specific activity = 2×10^9 to 4×10^9 cpm/μg). (**C**) Ligation of Pst I chromatin fragments in G1I cells. Nuclei isolated from G1I cells were subjected to the nuclear ligation assay (*11*). The DNA was purified and digested with Hind III, and 50 ng of purified G1I DNA plus 25 fg of Pst I PCR standard (*17*) were amplified by PCR (*24*) with the *PRL* primers I and II (*17*) with 2.5 mM $MgCl_2$. Southern blots were probed as described in (B). The blots were exposed to x-ray film for 5 to 15 min with one intensifying screen. Lane 1, ^{32}P-labeled pGem markers (Promega); lane 2, without Pst I and ligase; lane 3, with Pst I, without ligase; lane 4, without Pst I, with ligase; lane 5, with both Pst I and ligase. Solid bars show ligated Pst I chromatin fragments; open bars show Pst I chromatin fragments not ligated. (**D**) Ligation of Pst I chromatin fragments in GH_3 cells. Nuclei were isolated from GH_3 cells and then subjected to the nuclear ligation assay (*11*). Purified DNA was amplified by PCR and analyzed by Southern blotting as described in (C), except 1 μg of purified GH_3 DNA was used in each PCR reaction and no Pst I PCR standard was added. Lane 1, without Pst I and ligase; lane 2, with Pst I, without ligase; lane 3, without Pst I, with ligase; lane 4, with both Pst I and ligase. Sizes of DNA fragments are indicated to the right and left of the gels in (B), (C), and (D) in base pairs.

U/ml) for 10 min at 16°C, and then analyzed by the PCR-mediated method. The 1154-bp P_4-P_6 Pst I DNA fragment could be ligated (Fig. 3B, lanes 7 and 8) once the constraints of the chromatin structure were removed. These data suggest that unlike the 5′ upstream regulatory region of *PRL*, the Tn*5* gene is specifically packaged into a chromatin structure that does not allow interaction between widely separated regions.

Factors that interact with the 5′ distal enhancer region of *PRL* and stimulate promoter activity may stabilize the formation of chromatin loops. We treated G1I cells with estrogen and examined whether a higher level of chromatin loops between the 5′ distal enhancer and the proximal promoter was formed compared to control cells. Estrogen increased the ligation frequency (*15*) of P_3-P_4 and P_3-P_5 Pst I chromatin fragments two- to threefold compared to the control (Fig. 4), independent of the amount of 1941-bp P_3-P_4 chromatin fragment produced (*21*).

The close association of the distal enhancer with the proximal promoter appears to be intrinsic to the chromatin structure of the 5′ upstream regulatory region of *PRL* and probably reflects a specific folded state of the chromatin that is stabilized by estrogen treatment. Thus, the molecular mechanism by which estrogen enhances *PRL* transcription involves the ability of the hormone-occupied ER to stabilize the interaction between the enhancer and promoter regions of *PRL*, which would allow protein-protein interactions to occur between distal enhancer–binding proteins and promoter-binding proteins. This would result in a more stable preinitiation complex and augment the initiation of transcription by RNA polymerase II. Crenshaw *et al.* (*22*) showed that in transgenic mice high levels of *PRL* expression required synergistic interactions between the proximal promoter and the distal enhancer elements. We postulate that an intermediate chromatin structure between the 10-nm polynucleosome string and the 30-nm filament (*23*) may exist and allow widely separated transcription regulatory domains to communicate by direct protein-protein interactions, thus affecting the transcriptional activity of the gene. This loop structure may be intrinsic to the chromatin or "tethered" by specific protein factors.

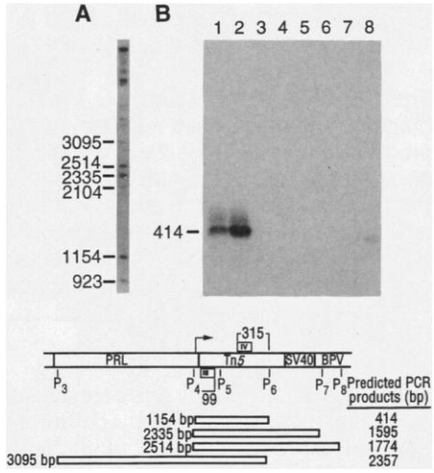

Fig. 3. Specificity of Pst I chromatin fragment ligation. (**A**) Southern blot of Pst I *PRL*-Tn*5*-BPV DNA fragments. The procedure was as described (Fig. 2B), except the blot was probed with a ^{32}P-labeled Pst I (+219) to Pst I (+1144) Tn*5* DNA fragment (P_5-P_6) (Fig. 4) (specific activity = 2×10^9 to 4×10^9 cpm/μg). (**B**) Ligation of Tn*5* Pst I chromatin fragments. Nuclei from G1I cells were subjected to the nuclear ligation assay (*11*). The DNA was purified, digested with Pvu II, and amplified by PCR (*24*) with the Tn*5* primers III and IV (*17*) with 1.5 mM $MgCl_2$. Southern blots of the PCR reaction products were probed with a ^{32}P-labeled Tn*5* DNA fragment (P_5-P_6). The specific activity of the probe as well as the hybridization and the x-ray film exposure conditions were identical to those in Fig. 2C. Lane 1, 0.02 fg of the 414-bp standard (*17*) plus 50 ng of GH_3 genomic DNA; lane 2, 0.1 fg of the 414-bp standard plus 50 ng of GH_3 genomic DNA; lane 3, with both Pst I and ligase (nuclear) and 50 ng of G1I DNA; lane 4, with both Pst I and ligase (nuclear) and 250 ng of G1I DNA; lane 5, with Pst I, without ligase (nuclear), and with 50 ng of G1I DNA; lane 6, with Pst I, without ligase (nuclear), and with 250 ng of G1I DNA; lane 7, with Pst I, without ligase (nuclear), with ligase (in vitro; 20 U/ml), and with 50 ng of G1I DNA; and lane 8, with Pst I, without ligase (nuclear), with ligase (in vitro; 100 U/ml), and with 50 ng of G1I DNA. Bars show Pst I chromatin fragments not nuclear-ligated. Ligation of Pst I chromatin fragments of 923 bp (P_5-P_6), 2104 bp (P_5-P_7), and 2283 bp (P_5-P_8) would not be detected with primers III and IV.

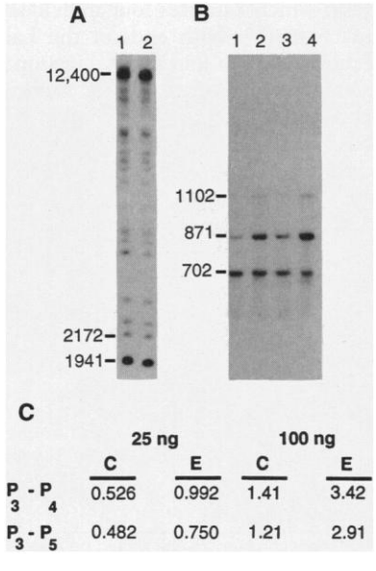

Fig. 4. Effect of estrogen on the ligation of Pst I *PRL* chromatin fragments. G1I cells were grown in low estrogen media (*13*) for 32 hours and then treated with either 10 nM 17β-estradiol or ethanol vehicle (control) for 16 hours. Nuclei were prepared and subjected to the nuclear ligation assay (*11*). (**A**) Southern blot of Pst I *PRL*-Tn*5*-BPV DNA fragments obtained from control and 17β-estradiol–treated cells. The procedure was as described (Fig. 2B). Lane 1, control; lane 2, 17β-estradiol. (**B**) Effect of 17β-estradiol on the ligation of Pst I *PRL* chromatin fragments. Twenty-five or 100 ng of purified G1I DNA plus 25 fg of Pst I *PRL* DNA PCR standard were amplified by PCR (*24*) with *PRL* primers I and II (*17*). The PCR reaction products were analyzed as described (Fig. 2C). Lane 1, 25 ng of G1I control; lane 2, 100 ng of G1I control; lane 3, 25 ng of G1I estrogen; lane 4, 100 ng of G1I estrogen. (**C**) Effect of estrogen on the ligation frequency of Pst I *PRL* chromatin fragments with either 25 ng or 100 ng of G1I DNA. The ligation frequency of Pst I *PRL* chromatin fragments was computed as described (*15*). C, control; E, estrogen added.

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11. In a nuclear ligation assay, isolated nuclei (*10*) were resuspended in 50 mM NaCl, 50 mM tris-Cl (pH 8.0), and 10 mM $MgCl_2$ at a concentration of 15 absorbance units at 260 nm (in 200 to 400 μl) and incubated with or without Pst I (500 U/ml) for 15 min at 37°C. To remove the Pst I, we washed the nuclei twice with 0.5 ml of ice-cold homogenizing buffer (*10*), resuspended them in 50 mM tris-Cl, 10 mM $MgCl_2$, 2 mM adenosine triphosphate, and 2 mM dithiothreitol at a concentration of 25 to 30 absorbance units at 260 nm, and then incubated them with or without T4 DNA ligase (BRL) (20 U/ml) for 10 min at 16°C. The ligation reaction was stopped by SDS–proteinase

K–EDTA and the DNA purified by phenol-chloroform extraction and ethanol precipitation.
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14. PCR products obtained from a nuclear ligation assay performed on G1I and GH_3 nuclei were digested with either Hinf I (G1I) or Hae III (GH_3) and then analyzed by Southern (DNA) blotting with a ^{32}P-labeled Hind III–Pst I *PRL* probe. Hinf I digestion of 871- and 1102-bp PCR products obtained from G1I nuclei formed the predicted (based on the known DNA sequence) 494- and 725-bp fragments, respectively. Hae III digestion of the 871- and 973-bp PCR products obtained from GH_3 nuclei resulted in the formation of three predicted fragments at 729, 627, and 244 bp. The formation of the unique Pst I junction (−1953 to −12 bp) by the ligation of the 5′ distal Pst I site of *PRL* at −1953 bp with the proximal promoter Pst I site at −12 bp was confirmed by dideoxynucleotide sequencing of the 871-bp PCR product.
15. To calculate the ligation frequency of the Pst I fragments, we scanned Southern blots of Pst I *PRL*-Tn*5*-BPV DNA fragments by a Hoefer laser densitometer, and the ratios of the 1941-bp and 2172-bp fragments compared to the full-length 12.4-kb Xba I fragment were determined. Bands corresponding to the 702-bp internal standard, the 871-bp P_3-P_4 Pst I DNA fragments, and the 1102-bp P_3-P_5 Pst I DNA fragments were excised from the nylon membrane and counted in a scintillation counter. The ligation frequency was determined by the following equation:

$$\frac{\dfrac{P_3\text{-}P_4 \text{ (or } P_3\text{-}P_5\text{) Pst I PCR fragment (cpm)}}{\text{PCR internal standard (cpm)}}}{\dfrac{\text{1941- (or 2172-) bp Pst I DNA fragment}}{\text{12.4-kb Xba I DNA fragment}}}$$

where the Pst I and Xba I DNA fragments are measured in scanning units.
16. For comparison, see Fig. 4C.
17. A Pst I DNA PCR standard used with *PRL* primers I and II (below) was prepared by digesting a 1941-bp Pst I *PRL* DNA fragment (P_3 to P_4) (Fig. 2) with Hae III (−1748) and then adding Pst I linkers to the ends of the DNA fragment. The 1772-bp Pst I fragment was gel purified, circularized with T4 DNA ligase, and then linearized with Hind III. The Hind III digestion products were amplified by PCR with *PRL* primers I [(−1495) 5′-GGAAGATATAGATAAATAAATCGTT-3′ (−1519)] and II [(−418) 5′-TTTAATTTACCCAATAATCTTGAAT-3′ (−394)], which yielded a 702-bp DNA fragment. We obtained the Pst I DNA PCR standard used with Tn*5* primers III and IV (below) by ligating in vitro an 1154-bp Pst I fragment from P_4 (−10) to P_6 (+1144) (Fig. 4) followed by linearization with Pvu II. The Pvu II digestion products were amplified by PCR with the Tn*5* primers III [(+89) 5′-TCCACCAAGCGGCCGGAGAACCTG-3′ (+65)] and IV [(+830) 5′-CTGAGCGGGACTCTGGGGTTCGAAATGACC-3′ (+859)], which produced a 414-bp DNA fragment. The 414-bp PCR product of Tn*5* primers III and IV and the 702-bp PCR product of *PRL* primers I and II were gel-purified and quantitated by comparison of ethidium bromide intensities on an agarose gel with known quantities of DNA. The 702-bp fragment was also used as an internal standard to monitor amplification efficiency (Fig. 4).
18. K. E. Cullen and M. A. Seyfred, unpublished results.
19. For comparison, see Fig. 4A.
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21. Estrogen appeared to have little or no effect on the formation of the 1941-bp P_3-P_4 chromatin fragment (Fig. 4). In other experiments, estrogen enhanced the formation of the 1941-bp P_3-P_4 chromatin fragment, which resulted in a corresponding increase in the amount of P_3-P_4 ligation products formed. Upon correction for the efficiency of the PCR amplification and the amount of substrate available for ligation, estrogen increased the ligation frequency two- to threefold compared to the control.
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24. Pst I ligation fragments were amplified by PCR with primer (0.5 μM each) in 50 mM KCl, 10 mM tris-Cl (pH 8.3), 0.01% gelatin, 1.5 or 2.5 mM $MgCl_2$ (optimized for each primer set), and Taq polymerase (20 U/ml) (Boehringer Mannheim Biochemicals) in 100 μl of reaction volumes. Samples were preheated for 5 min at 95°C, placed on ice for 5 min, and then subjected to cycling conditions of 94°C for 60 s, 55°C for 30 s, and 72°C for 30 s for three cycles followed by 27 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 15 s. A 25-μl aliquot of the PCR reaction was analyzed on a 1.2% agarose gel, blotted to nylon, and then hybridized with a specific ^{32}P-labeled DNA probe.
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An NAD Derivative Produced During Transfer RNA Splicing: ADP-Ribose 1″-2″ Cyclic Phosphate

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Transfer RNA (tRNA) splicing is essential in *Saccharomyces cerevisiae* as well as in humans, and many of its features are the same in both. In yeast, the final step of this process is removal of the 2′ phosphate generated at the splice junction during ligation. A nicotinamide adenine dinucleotide (NAD)–dependent phosphotransferase catalyzes removal of the 2′ phosphate and produces a small molecule. It is shown here that this small molecule is an NAD derivative: adenosine diphosphate (ADP)–ribose 1″-2″ cyclic phosphate. Evidence is also presented that this molecule is produced in *Xenopus laevis* oocytes as a result of dephosphorylation of ligated tRNA.

Intron-containing tRNA genes are ubiquitous in the nuclei of eukaryotes (*1*). Introns invariably occur one base 3′ of the anticodon, and intron removal is an essential event. Transfer RNA splicing is best understood in *S. cerevisiae*. Splicing there is initiated by an endonuclease that recognizes precursor tRNA (pre-tRNA) structure, measures the length of the anticodon stem to locate the intron, and excises the intron to generate two half-molecules (*2*). These half-molecules are substrates for tRNA ligase, which catalyzes four individual steps to restructure both ends of the half-molecules and then join them. Ligation generates a mature-length tRNA bearing a 2′ phosphate at the splice junction (*3*). An NAD-dependent phosphotransferase that catalyzes removal of the splice junction 2′ phosphate has been identified in yeast (*4*, *5*). This enzyme efficiently removes an internal 2′ phosphate from tRNA or from an oligonucleotide as small as a dimer; however, it will not detectably remove a terminal 5′, 3′, 2′, or 2′-3′ cyclic phosphate from an oligonucleotide (*4*, *6*). Dephosphorylation of ligated tRNA in vitro is accompanied by transfer of the 2′ phosphate to an acceptor molecule, which forms an unusual small molecule that we call the phosphotransfer product. We show here that the phosphotransfer product is an NAD derivative: ADP-ribose 1″-2″ cyclic phosphate (Fig. 1). This phosphotransfer product is also made in *Xenopus* oocytes, which indicates that dephosphorylation of ligated tRNA occurs by the same process in vivo.

To elucidate the structure of the phosphotransfer product, we treated it with various enzymes. Phosphotransfer product bearing a radiolabeled transferred phosphate can be generated, after endonucleolytic cleavage, ligation, and phosphotransfer (*5*), from a pre-tRNA^{Phe} transcript labeled with [α-^{32}P]adenosine triphosphate (ATP). Surprisingly, the purified phosphotransfer product was resistant to hydrolysis with calf intestinal phosphatase (Fig. 2, lane 2) and bacterial alkaline phosphatase. This phosphatase resistance is highly unusual, as both phosphatases are nonspecific and would be expected to remove any O-linked phosphomonoester (*7*). Although the molecule was pyrophosphatase sensitive (Fig. 2, lane 3), subsequent treatment with

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