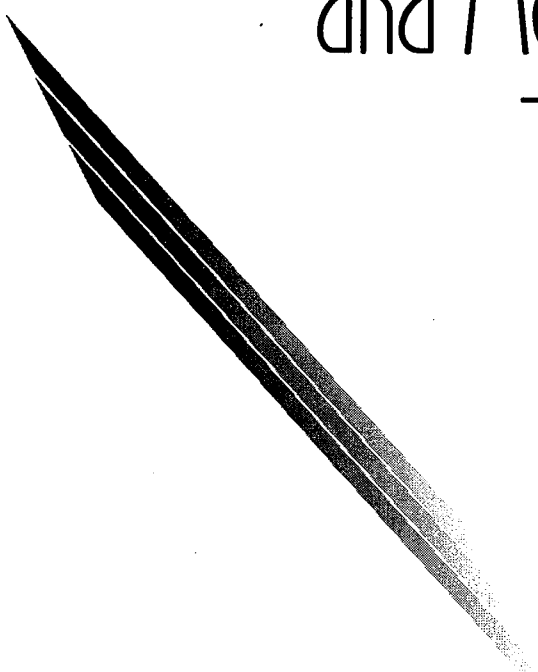
RUSSIAN ACADEMY OF SCIENCES
ST. PETERSBURG SCIENTIFIC CENTER RAS
B. P. KONSTANTINOV PETERSBURG NUCLEAR PHYSICS INSTITUTE RAS

Bresler Memorial Lectures

Molecular Genetics Biophysics and Medicine Today



St. Petersburg
2002

Oxidation of Thiol Compounds with Molecular Oxygen. Stoichiometry, Kinetics and Reaction Mechanisms

H.A. Bagiyan, I.K. Koroleva, N.V. Soroka, and A.V. Ufimtsev

Division of Molecular and Radiation Biophysics, B. P. Konstantinov Petersburg Nuclear Physics Institute, Russian Academy of Sciences, Gatchina, 188300, Russia.

E-mail: heinrich@omrb.pnpi.spb.ru

Over 25 years ago in our research team were completed extensive studies on the effect of thiol compounds structure on their oxidation in water solutions. It was completed by establishing the mechanism of catalytic oxidation that allowed quantitative description of behavior of certain thiol compound in reaction with oxygen in the presence of copper ions. Since the results of the studies provided important practical opportunities, namely, purification of petroleum products from mercaptanes, the results could not be published in full. The recent publications touching this problem show that the results of our investigations are still interesting and useful for specialists as they have been 25 year before.

These studies were actively supported by Professor Bresler, and without his management of the applied aspects of the work we would hardly perform the industrial trials of our catalytic systems.

In the course of study we revealed that the background oxidation (autooxidation) of thiol compounds actually was effected by the impurity of variable-valence metals. A number of analytical and preparative methods were developed that served as basis for thoroughly investigating of thiol compounds catalytic oxidation in the presence of copper ions. It was established that thiol compounds are oxidized into the corresponding disulfides whereas oxygen is transformed into hydrogen peroxide or water (depending on the structure of the oxidized thiol compound) within the coordination sphere of copper. It was shown that in keeping with the kinetic characteristics of the catalysed oxidation and depending on the chelating ability of thiol compounds the oxidation of the latter followed two different mechanisms. They were substantiated with the use of our previously obtained data on complexing of thiol compound with Cu^+. The accelerating effect in oxidation of mercaptanes at introduction into sistem (mercaptane + Cu^+) in water solution of small amounts of chelating thiol compounds revealed in the study was applied to development of the method of petroleum products purification from mercaptanes, and the modification of the method was used for natural gas separation from hydrogen sulfide.

Май 2002 г.

Molecular Basis of Tn*5* Transposition

William S. Reznikoff

Department of Biochemistry
University of Wisconsin,
433 Babcock Dr.,
Madison, WI, 53706, U. S. A.
E-mail: reznikoff@biochem.wisc.edu

Tn*5* is a bacterial transposable element that transposes via a cut and paste mechanism. Because of recent genetic, biochemical and structural discoveries, more is known about the Tn*5* transposition process than for other similar processes. This chapter reviews our understanding of Tn*5* transposition and briefly mentions how the Tn*5* system has been used to study basic questions involving intracellular DNA structure and has been used as a tool in biotechnology.

Key words: Tn*5*, transposon, transposition, transposase.

Introduction

We can now predict that we will likely achieve a detailed molecular understanding of Tn*5* transposition within the next few years. Dr. Simon Bresler planted many of the seeds that led to this achievement. His seeds included initiating experiments that recognized the power of transposable elements as experimental tools, an interest in the genetic mechanisms of transposition within a larger framework of studying the three R's (replication, recombination and repair), and the mentoring and stimulating of younger scientists to continue studying this fascinating subject. I never had the honor of meeting Dr. Bresler, but I have been a close colleague of two of his protégés, Drs. Vladislav Lanzov and Igor Goryshin. This relationship has led to many wonderful personal and professional experiences.

The value of Tn*5* research extends far beyond the elucidation of how this transposon orchestrates its genetic movement. We now know that the Tn*5* transposase is a member of the RnaseH super-family of proteins that includes bacterial transposases, retroviral integrases and probably RAG-1 [1, 2]. All of these proteins share a tertiary structure architecture and the same active site structure, and have mechanistic similarities. However, the Tn*5* transposase is the best defined, thus, for the moment, it is a surrogate for studying the other proteins.

Tn*5* transposition is also a powerful tool for molecular genetics and biotechnology research. I will end my chapter with a brief overview of how Tn*5* work has and will contribute to these fields.

The Tn*5* transposition mechanism

Our current understanding of Tn*5* transposition is described in outline form in Fig. 1. The key macromolecular participants are the transposase, a 476 amino acid protein, the transposon DNA that is defined by two 19 bp inverted sequences at its ends, and target DNA. No host functions are required until transposase needs to be disengaged from the product molecule [3]. Below the known steps in Tn*5* transposition will be described some detail.

Formation of the synaptic complex

The first observed step involves synaptic complex formation [4]. The synaptic complex formation is typically modeled as occurring in two steps: monomeric transposase binding to the DNA end sequences followed by dimerization of the monomeric transposase-DNA complexes, but, in fact, we do not know which step occurs first. Alternative possibilities are that transposase dimerization could occur first followed by dimerization or both sequences could occur (see Fig. 2). The key research problem is that we can not detect either full-length transposase binding to DNA [5] or full-length transposase dimerization [6, 7] as separate steps. This is related to a key down regulatory mechanism in Tn*5* transposition (see below).

Transposase binding to DNA and transposase dimerization both require a substantial protein conformational change in order to expose the DNA binding determinants in the N-terminus and the dimerization determinant in the C-terminus [8]. Otherwise the C-terminus and the N-terminus interact with each other to block their respective activities. Experimentally the DNA binding can not be observed unless the C-terminal sequences are removed or otherwise occupied [9] and the dimerization can not be observed unless the N-terminal sequences are removed or otherwise occupied [6]. This C-terminal-N-terminal inhibition may be biologically important. In order to ensure survival of the host, the frequency of transposition is severely down regulated and the inactivity of the transposase resulting from this inhibition contributes substantially to this transposase inactivation. In addition the C-terminal-N-terminal inhibition may be the basis of the strong *cis* bias of wild type transposase action *in vivo*; the ribosome associated nascent transposase may initiate transposition by binding to a nearby end prior to translation of the C-terminus.

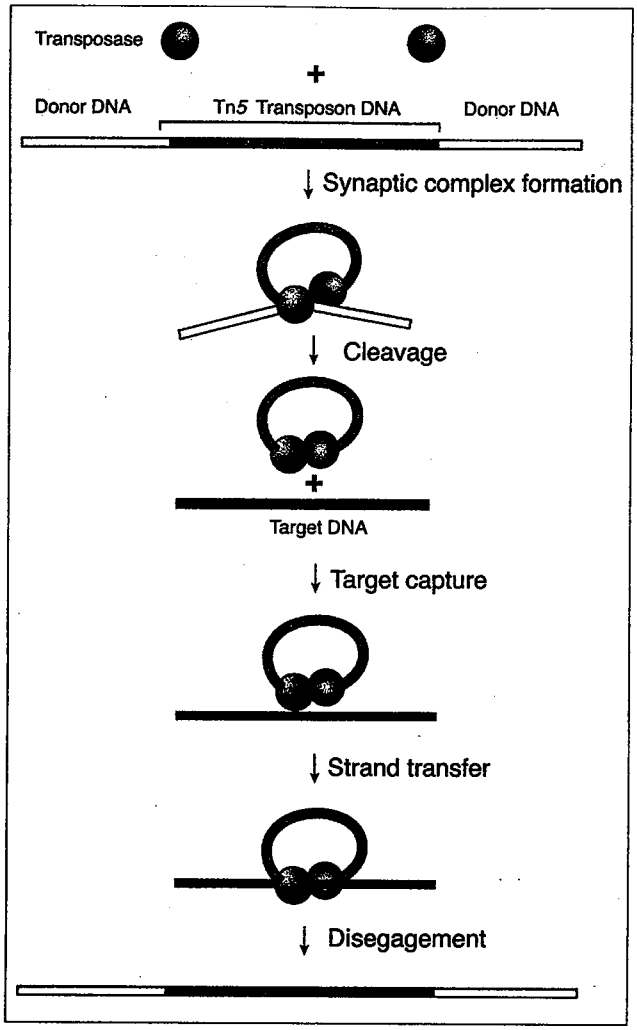


Figure 1. Tn5 transposition pathway.

The process through which Tn5 transposes is presented schematically. First two molecules of the transposase protein interacts with the transposon end sequences to form the synaptic complex. Formation of the synaptic complex might occur through two different mechanisms as described in Fig. 2. Next the transposase in the presence of Mg^{++} catalyzes the DNA cleavage events that results in transposon DNA release from donor DNA. The mechanism for this catalysis is described in Fig. 4. A structure of the released synaptic complex that follows DNA cleavage is shown in Fig. 2. Following DNA cleavage the released synaptic complex binds to target DNA. After target capture the transposase catalyzes strand transfer into the target in which the 3'OH ends attack phosphodiester bonds that are spaced 9 base pairs apart. The strand transfer complex is then disengaged with the aid of an unknown host function.

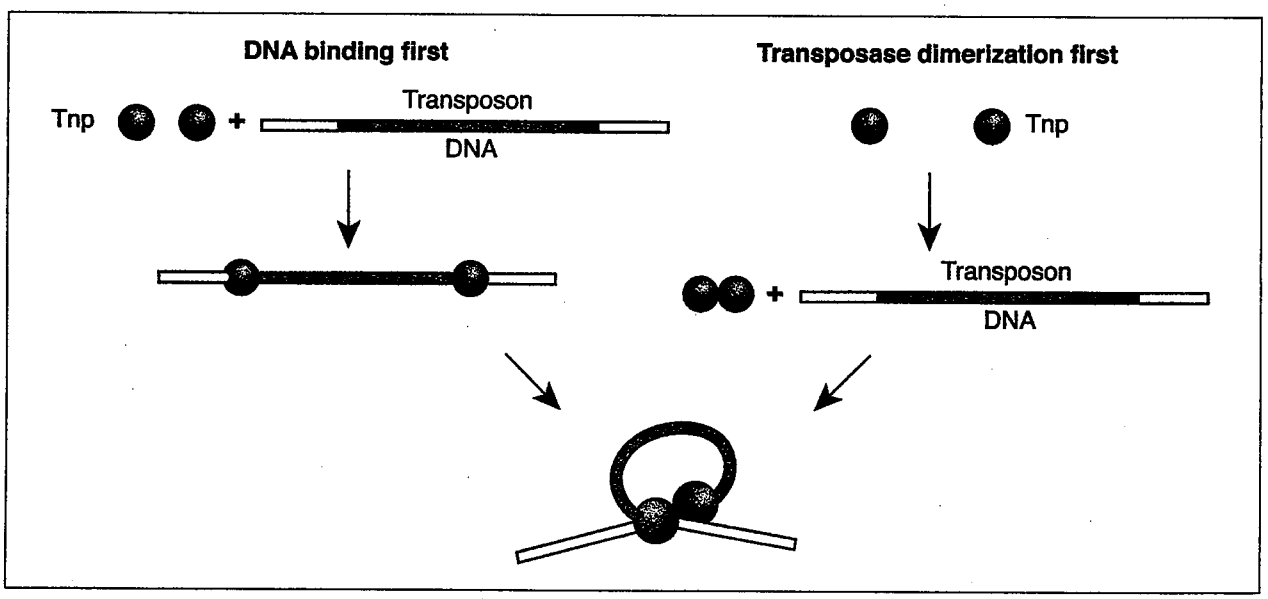


Figure 2. Alternate pathways to synaptic complex formation.

The sequence of events leading to synapsis is not known. There are three options. Either the transposase (Tnp) binds to the transposon ends first and then dimerizes, or dimerization occurs first followed by DNA binding, or both pathways function.

Nonetheless we have an excellent idea of the transposase-DNA interactions involved in this first critical step in transposition. Three types of studies were used to study these contacts. First, genetic experiments resulted in the isolation of a variety of transposase mutations that inhibited, enhanced or altered the specificity of end binding [7, 10, 11]. Second, monomeric transposase-end DNA binding was studied utilizing a C-terminal deleted version of the transposase [9]. This allowed a variety of biochemical experiments such as gel retardation, missing nucleoside and OH radical protection studies [4]. Finally, the structure of a transposase-end DNA synaptic complex (the product of the DNA cleavage catalytic steps) has been determined (Fig. 3) [2]. This structure (confirming and extending the genetic and biochemical experiments) indicated the key transposase residues involved in initial or *cis* end DNA binding. The key transposase residues required for end DNA binding are known to be located in the N-terminus (residue 26 to residue 61) and secondarily in residues 344-348. These residues are involved in contacting positions in the end sequence somewhat distal to cleavage site; from bp 6 through to 18.

Dimerization of transposase (or the transposase-DNA complexes) has been studied by an examination of the dimerization of the N-terminal truncated transposase and by looking at the structure of the final transposase-DNA synaptic complexes [2, 6, 12]. Dimerization is a critical step in transposition since DNA catalysis occurs *in trans* [13]. That is the DNA initially bound by one transposase is cleaved by the other transposase.

The protein-protein contacts in the dimer are quite modest involving hydrophobic contacts between the two C-terminal alpha helices [1, 2]. Genetic studies have confirmed that residues G462 and A466 are, in fact, critical for dimerization and transposition [6, 12]. More interesting is that the dimeric synaptic complexes display extensive *trans* transposase-DNA contacts involving residues around the active site as well as residues 243-253 [2]. These latter residues resemble a flap that appears to have folded over the DNA after dimerization has occurred [8]. The DNA regions contacted by the transposase *in trans* includes positions 1-7 [2, 4].

We do not yet have a structure of this first synaptic complex, i.e. a synaptic complex in which donor DNA is still attached to the transposon end DNA sequences. But based upon the nature of the catalytic steps that follow and subsequent structures that we have determined this first synaptic complex must have a substantial distortion of the DNA surrounding the DNA cleavage site.

DNA cleavage catalysis

The DNA cleavage that removes the donor DNA is known to occur via a three step process: nicking of the transferred strand-donor junction to yield a free 3' OH group, attack of the 3'OH end onto the opposite strand to generate a DNA hairpin, and cleavage of the hairpin (Fig. 4) [14]. This catalytic mechanism has also been studied in detail for the related Tn*10* transposition system [15, 16]. The catalysis involves two Mg (or Mn) ions bound to the active site DDE residues activating oxygens to be nucleophiles onto phosphate groups through a chemistry similar to that proposed for DNA polymerase [17, 18].

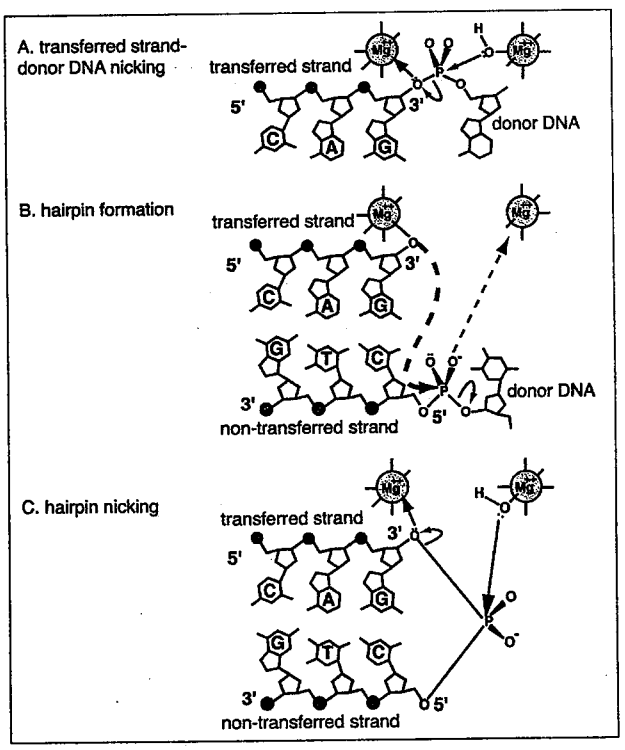


Figure 4. Mechanism of DNA cleavage.

Based upon the observed presence of two metals in the active site and the stereochemistry of the DNA nicking and strand transfer reactions [17 and 16], a mechanism for the DNA cleavage steps has been proposed [17] that is consistent with similar chemistries described previously [18]. This figure is similar to one presented previously [17].

Figure 3. Molecular structure of the Tn5 synaptic complex.

X-ray crystallography was used to determine the molecular structure of hyperactive Tn5 transposase bound to 20 base pair hyperactive end DNA sequences. The structure is similar to that presented before [2]. The two monomers of transposase are presented in yellow and blue and the DNAs in purple. The active site DDE residues ant the Mg^{++} are in red. The yellow monomer has bound to the DNA on the right and appears to have inserted the tip of the DNA (where cleavage has occurred) into the active site of the blue monomer. Likewise the blue monomer of transposase appears to have inserted its DNA into the active site of the yellow monomer. This structure is compatible with genetic analyses of the DNA binding domains, the active site residues and the dimerization domain. The structure also reflects the *trans* nature of the catalysis.

The first catalytic step (hairpin formation) must involve DNA distortion since base pairs 1 and 2 must be denatured and the 3' and 5' ends end up substantially closer together than they were in the intact duplex. A key event facilitating this distortion maybe the flipping of a thymine at position 2 of the non-transferred strand out of the helix into a protein pocket [19]. Other protein-DNA interactions that result in DNA distortions also have a similar flipped base [20]. A tryptophan to alanine mutation, that removes a key stacking relationship to this position 2 thymine, inhibits hairpin formation [19].

The transposase-hairpin structure has recently been determined (Lovell, Goryshin, Reznikoff and Rayment, unpublished). It maintains the flipped base at position 2 plus it has the position one bases disoriented. Otherwise the structure is strikingly similar to that shown for the synaptic complex shown in Fig. 3.

There are several key residues in addition to the DDE residues that are conserved in many transposases and retroviral integrases. These residues are shown to make interesting DNA contacts in the crystal structure and mutations in these residues typically block one or more catalytic steps [2, 21].

Target DNA binding and strand transfer

The next steps in the transposition process involve the binding of the transposase to the target DNA sequence followed by strand transfer catalysis. Target binding involves the precise localization of the target DNA sequence within the transposase-transposon synaptic complex so that the transposon 3' OH ends are directed at the target scissile phosphates 9 bp apart. We know a considerable amount about the target sequence specificity of this event thanks largely to the work of Drs. Goryshin, Kil and Lanzov [22, see below], but little about the transposase residues involved.

Following strand transfer, the 9 bp target sequence is duplicated at each end of the inserted transposon. Thus to determine the sequence specificity of target binding we sequenced the 9 bp duplications and adjacent sequences of two populations of independent Tn*5* transposition products in one 660 bp target gene and then attempted to construct a consensus target sequence. One of these collections was from *in vivo* experiments and one was from *in vitro*. The results from the two populations were the same thus there is no host function that appears to play an important role in determining target specificity. The consensus sequence was AGNTYWRANCT in which the outside base pairs represent specificity contributed by the sequence adjacent to the 9 bp target. Two factors were of note. The specificity of none of the 11 indicated sites was absolute meaning that any of the four possible base pairs could be accepted at any site thus the consensus and therefore the specificity was relatively weak. Second the location of several of the sites appeared to be clustered in overlapping arrays. An elegant model that explains this is that perhaps the synaptic complexes form microfilaments on target DNA prior to strand transfer and that the precise synaptic complex within the array that catalyzes strand transfer is randomly located.

Even though Tn*5* transposition appears to have some target specificity, the target specificity is sufficiently weak so that on a genome wide basis, Tn*5* appears to transpose essentially randomly (J. Jendrisak, personal communication; S. Gerdes, personal communication). That is, inserts in several positions can be found relatively easily and with essentially equivalent probabilities.

No direct evidence has been presented as to which transposase residues play a role in target binding. However, careful examination of the synaptic complex structure reveals that the 3' OH ends of the transposon DNA are imbedded approximately 41 Angstroms apart within a cleft [2]. This cleft has about the right dimensions to fit a duplex DNA and has a large number of basic side chains [2, 21].

Following binding of the target, catalysis of strand transfer occurs via a Mg^{++} (or Mn^{++}) aided attack by the two transposon 3'OH groups onto the target scissile phosphates. This attack, at least *in vitro*, is not concerted since it is easy to detect single end transfer products [21].

Strand Transfer Complex Dissociation

Another key aspect of Tn*5* transposition is that the strand transfer complexes do not self dissociate [3]. In other words the transposase does not turn over and thus is not a true enzyme. *In vitro* we dissociate the complexes using some sort of protein denaturation technique such as phenol extraction or heating in the presence of detergent. But *in vivo*, some sort of host factor(s) must be involved. This factor must be present in essentially all organisms because Tn*5* transposition can occur in all tested bacteria [23]. My hypothesis is that the critical factor(s) are trypsin like proteases. This hypothesis is based upon the following observations. First, cells that synthesize transposase under all observed circumstances always contain N-terminal cleaved products [24]. These peptides are generated by cleavage immediately adjacent to residues R30, K40 and R62. Cleavage at these sites disrupts the critical *cis* DNA binding domain and thus should disengage transposase from the strand transfer complexes. Mutations of residues 30 and 62 to glutamine result in inactive transposase and thus are uninformative. Mutation K40Q has a very unique phenotype [25]. The transposase has only 1% activity *in vivo* but is nearly fully active *in vitro*. The simplest interpretation of these observations is that the K40Q mutation has impaired the normal *in vivo* strand transfer complex dissociation mechanism. While these results do not rule out the role of some chaperone in dissociation as is observed for the Mu system [26], they suggest that the host fact may be a protease.

Tn*5* as a tool for studying basic molecular questions

Many studies of Tn*5* transposition have addressed important issues in molecular biology. Two examples are: What is the helical DNA repeat *in vivo*? What is the DNA persistence length *in vivo*? These issues were addressed in some elegant experiments described by Drs. Goryshin and Kil [27]. Essentially the transposition frequency was determined for a series of constructs that varied the distance between the transposon ends. Constructs with distances less than 64 base pairs did not give transposition products. Thus the *in vivo* persistence length of DNA under the growth conditions used is less than, or equal to, 64 bp. Between 64 and 175 base pairs the transposition frequency oscillated with a periodicity of 10.5 bp. This is a reflection of the *in vivo* helical repeat for the set of DNA sequences that were tested.

Tn*5* as a tool in biotechnology

Transposition in general and Tn*5* transposition in particular has long been a powerful tool for the microbial geneticist. During the last few years, with the advent of *in vitro* transposition systems, a number of powerful Tn*5* related tools have been introduced to the researcher that will have major impacts on the way that we perform genome analysis and manipulation.

A key property of the Tn*5* transposition system is that the only macromolecular requirements are the transposase, a transposon DNA defined by specific 19 base pair end sequences, and the target DNA [3, 8]. Except for the 19 base pair end sequences, the transposon DNA can contain any desired sequence although there are length constraints (the DNA must be longer than the DNA persistence length and the frequency of transposition drops off after the transposon exceeds a few thousand base pairs in length). Examples of DNA sequences that can be carried within a transposon include: selectable markers, primer binding sequences for DNA sequencing protocols, epitope encoding sequences for performing protein structure/function studies, reporter functions for gene or operon expression studies, promoters, site specific recognition sites, and inside out transposons.

Dr. Igor Goryshin, a protégée of Dr. Bresler, made an important discovery for many of the applications. Tn*5* transposase-transposon synaptic complexes are extremely stable (in the absence of Mg^{++} or Mn^{++}) and can be electroporated into bacterial cells. After entry into the cells, the complexes then proceed with the transposition process [23]. This procedure avoids the complication of designing special vehicles for each type of target cell and generates a high yield of transposition events in an essentially synchronous fashion.

A major application for Tn*5* transposition systems is their use as a sequencing tool (distributing two primer binding sites throughout a target DNA) [8]. The use of a transposon carrying a second inside out transposon results in the transposon being a mechanism for generating adjacent deletions (or inversions) [28]. This has been used to generate nested deletions in protein coding genes or in DNAs to be sequenced [28], or to generate deletions within target genomes (I. Goryshin, T. Naumann, & W. Reznikoff, unpublished). An additional use Tn*5* transposition system is in the generation of random DNA fusions encoding, for example, RNA or protein fusions (T. Naumann, I. Goryshin, & W. Reznikoff, unpublished).

Conclusion

Dr. Bresler's training of imaginative and talented scientists and his vision of transposition as an interesting topic of investigation and as a source of genetic tools have certainly borne fruit. Tn*5* has provided a powerful window for investigating phosphoryl transfer reactions that change the structure of DNA. The tools that use this chemistry have provided important techniques for the current biotechnology revolution.

Acknowledgements

I thank Dr. Igor Goryshin and the other members of my laboratory for their research work, and Drs. Davies, Lovell and Rayment for the X-ray crystallographic analyses. The research was supported by grants from the National Institutes of Health (GM50692) and the National Science Foundation (MCB-84089).

References

1. D.R. Davies, L.M. Braam, W.S. Reznikoff, and I. Rayment (1999). The three-dimensional structure of a Tn*5* transposase-related protein determined to 2.9Å resolution. *J. Biol. Chem.* **274:** 11904-11913.

2*. D.R. Davies, I.Y. Goryshin, W.S. Reznikoff, and I. Rayment (2000). Three-dimensional structure on the Tn5 synaptic complex transposition intermediate. *Science* **289:** 77-85.

The paper is particularly interesting because it provides the only co-crystallographic analysis of a transposase-transposon DNA synaptic complex. This structure is compatible with all biochemical and genetic experiments and thus is physiologically relevant. The structure provides a detailed picture of how transposition occurs.

3. I.Y. Goryshin and W.S. Reznikoff (1998). Tn*5* *in vitro* transposition. *J. Biol. Chem.* **273:** 7367-7374.

4. A. Bhasin, I.Y. Goryshin, M. Steiniger-White, D. York, and W.S. Reznikoff (2000). Characterization of a Tn*5* pre-cleavage synaptic complex. *J. Mol. Biol.* **302:** 49-63.

5. D. York and W.S. Reznikoff (1997). DNA-binding and phasing analyses of Tn*5* transposase and a monomeric variant. *Nucleic Acids Res.* **25:** 2153-2160.

6. L.M. Braam, I.Y. Goryshin, and W.S. Reznikoff (1999). The mechanism of Tn*5* inhibition: carboxyl-terminal dimerization. *J. Biol. Chem.* **274:** 86-92.

7. M.D. Weinreich, L. Mahnke-Braam, and W.S. Reznikoff (1994) A functional analysis of the Tn5 transposase: Identification of domains required for DNA binding and multimerization. *J. Mol. Biol.* **241:** 166-177.

8. W.S. Reznikoff (2002). Tn5 transposition. *in* Mobile DNA II (N. Craig, R. Craigie, M. Gellert, and A.M. Lambowitz, Eds.), pp. 403-421. American Society of Microbiology.

9. D. York and W.S. Reznikoff (1996). Purification and biochemical analysis of a monomeric form of Tn*5* transposase. *Nucleic Acids Res.* **24:** 3790-3796.

10. T.A. Naumann and W.S. Reznikoff (2002). Tn5 transposase with an altered specificity to transposon ends. *J. Bact.* **184:** 233-240.

11. M. Zhou and W.S. Reznikoff (1997). Tn*5* transposase mutants that alter DNA binding specificity. *J. Mol. Biol.* **271:** 362-373.

12. M. Steiniger-White and W.S. Reznikoff (2000). The C-terminal alpha helix of Tn5 transposase is required for synaptic complex formation. *J. Biol. Chem.* **275:** 23127-23133.

13. T.A. Naumann and W.S. Reznikoff (2000). *Trans* catalysis in Tn*5* transposition. *Proc. Natl. Acad. Sci. USA* **97:** 8944-8949.

14. A. Bhasin, I.Y. Goryshin, and W.S. Reznikoff (1999). Hairpin formation in Tn*5* transposition. *J. Biol. Chem.* **274:** 37021-37029.

15. A.K. Kennedy, A. Guhathakurta, N. Kleckner, and D.B. Haniford (1998). Tn*10* transposition via a DNA hairpin intermediate. *Cell* **95:** 125-134.

16. A.K. Kennedy, D.B. Haniford, and K. Mizuuchi (2000). Single active site catalysis of the successive phosphoryl transfer steps by DNA transposases: insights from phosphorothioate stereoselectivity. *Cell* **101:** 295-305.

17. S. Lovell, I.Y. Goryshin, W.S. Reznikoff, and I. Rayment (2002). Two metal active site binding of a Tn5 transposase synaptic complex. *Nat. Struct. Biol.* **9:** 278-281.

18. L.S. Beese and T.A. Steitz (1991). Structural basis for the 3'-5' exonuclease activity of *E. coli* DNA polymerase I: a two metal ion mechanism. *EMBO J.* **10:** 25-33.

19. B. Ason and W.S. Reznikoff (2002). Mutational analysis of the base flipping event found in Tn5 transposition. *J. Biol. Chem.* **277:** 11284-11291.

20. R.J. Roberts and X. Cheng (1998). Base flipping. *Ann. Rev. Biochem.* **67:** 181-198.

21. T.A. Naumann and W.S. Reznikoff (2002). Tn5 transposase active site mutants. *J. Biol. Chem.*: In press.

22. I.Y. Goryshin, J.A. Miller, Y.V. Kil, V.A. Lanzov, and W.S. Reznikoff (1998). Tn*5*/IS*50* target recognition. *Proc. Natl. Acad. Sci. USA* **95:** 10716-10721.

23*. I.Y. Goryshin, J. Jendrisak, L.M. Hoffman, R. Meis, and W.S. Reznikoff (2000). Insertional transposon mutagenesis by electroporation of released Tn5 transposition complexes. *Nature Biotech.* **18:** 97-100.

The paper describes a revolutionary new technology for introducing transposition complexes into different microorganisms.

24. L.M. Braam and W.S. Reznikoff (1998). Functional characterization of the Tn*5* transposase by limited proteolysis. *J. Biol. Chem.* **273:** 10908-10913.

25. S.S. Twining, I.Y. Goryshin, A. Bhasin, and W.S. Reznikoff (2001). Functional characterization of arginine 30, lysine 40 and arginine 62 in Tn5 transposase. *J. Biol. Chem.* **276:** 23135-23143.

26. I. Levchenko, L. Luo, and T.A. Baker (1995). Disassembly of the Mu transposase tetramer by the ClpX chaperone. *Genes Dev.* **9:** 2399-2408.

27. I.Y. Goryshin, Y.V. Kil, and W.S. Reznikoff (1994). DNA length, bending, and twisting constraints on IS50 transposition. *Proc. Natl. Acad. Sci. USA* **91:** 10834-10838.

28. D. York, J. Welch, I.Y. Goryshin, and W.S. Reznikoff (1998). Simple and efficient generation *in vitro* of nested deletions and inversions: Tn*5* intramolecular transposition. *Nucleic Acids Res.* **26:** 1927-1933.

Май 2002 г.