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RECENT ADVANCES IN THE DEVELOPMENT OF DNA HYBRIDIZATION PROBES FOR THE DETECTION AND CHARACTERIZATION OF *TRYPANOSOMA CRUZI*

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The development of sensitive, rapid and reliable methods for the detection of *T. cruzi* in the invertebrate or vertebrate hosts, as well as for the characterization of isolates, strains and clones of this parasite would represent a major advance for diagnostic, clinical and epidemiological studies in Chagas' disease. Currently xenodiagnosis, despite all its disadvantages, is still the only reliable direct parasitological method for diagnosing chronic Chagas' disease, while the present biochemical methods for parasite characterization include a step of population amplification which can lead to serious misinterpretations due to subpopulation selection (Gonçalves et al., 1984; Deane et al., 1984).

Nucleic acid hybridization is one of the major tools used by molecular biologists to detect, identify and quantitate DNA sequences or their specific transcripts. The development of recombinant DNA technology has made possible the isolation and amplification of defined DNA fragments which have been used as powerful and specific molecular probes to detect complementary nucleotide sequences. The development of probes which would be specific and sensitive for pathogen detection is nowadays an extremely active field of research (for review, see: Engleberg & Eisenstein, 1984). The definition and preparation of such DNA sequences and the development of methods for their non-radioactive labelling (Langer, Waldrop & Ward, 1981; Leary, Brigati & Ward, 1983; Renz & Kurz, 1984) will soon allow the routine use of probe technology in clinical laboratories thereby introducing a major change in current approaches to the detection and characterization of pathogenic microorganisms.

The occurrence of abundant, repetitive DNA sequences both in the nuclear and in the kinetoplast genomes of *Trypanosoma cruzi* has made possible the development of methods for the detection and characterization of this parasite through probe technology. In this symposium we will briefly discuss the current approaches used and the potential use of DNA probes in diagnostic, clinical and epidemiological studies in Chagas' disease.

Lizardi, Nogueira and collaborators isolated genomic clones of *T. cruzi*, containing members of a tandemly repeated nuclear DNA family, which were used as probes for parasite detection (Gonzales et al., 1984). Using radioactive phosphorus labelling the authors demonstrated the specificity of the probe for *T. cruzi* sequences (only *Leptomonas collosoma* gave a weak cross-hybridization signal) and they could detect as few as 30 parasites in blood samples of acutely-infected mice.

Frasch and collaborators have used whole kinetoplast DNA or cloned kinetoplast DNA minicircles in Southern-or dot-blot hybridization procedures for the identification and characterization of *T. cruzi* isolates (Sanchez et al., 1984). In addition to the detection of parasites, their probes were also useful for the typing of strains when used under high stringency hybridization conditions.

Fig. 1 shows an example of the use of total kinetoplast DNA labelled with radioactive phosphorus as a molecular probe for the characterization of *T. cruzi* strains. In this experiment kDNAs isolated from 65 different *T. cruzi* isolates were spotted on nitrocellulose filters and hybridized with Y or CL kDNA probes using the dot-blot hybridization technique of Kafatos, Jones & Efstratiadis, 1979. This type of experiment allows the characterization of the samples of "Y-like" or "CL-like" according to the intensity of hybridization signals. Strong hybridization signals with both probes can either be due to the presence of heterogeneous populations (Deane et al., 1984; Gonçalves et al., 1984) or to parasites at an intermediate evolutionary position between both probes. This approach generates a classification of *T. cruzi* strains based on the relationship of kDNA sequences, as does schizodeme analysis (Morel et al., 1980).

Synthetic DNA probes have several advantages over cloned DNA fragments for the detection of specific sequences: they have an extremely specific hybridization behaviour, can be labelled to very high specific activities, and can be prepared in large amounts by automated synthesis (Itakura, Rossi & Wallace, 1984). We have recently developed chemically synthesized short oligodeoxynucleotide probes for *T. cruzi* detection (Morel, Simpson, Grunstein & Simpson, in preparation). Based on our results of *T. cruzi* characterization by restriction fingerprinting of kinetoplast DNA (Morel et al., 1980) and on sequence data on cloned minicircle DNA (Gonçalves et al., 1984; Van Heuverswyn, Degrave, Mueller & Morel, unpublished) we selected specific sequences of the conserved, repeated regions of *T. cruzi* minicircles which could be of

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potential use in parasite detection. Three oligodeoxynucleotides of 21 bases each were synthesized in an automated DNA synthesizer, purified by sequencing gel electrophoresis and C18 SEP-PAK chromatography, labelled by T4 polynucleotide kinase and tested as probes for *T. cruzi* detection in dot-blot and Southern transfer assays. Our results showed that these probes could detect all strains tested, did not react with the controls (*H. samuelpessoai* or *Leishmania tarentolae* kDNA) and originated strong hybridization signals in Southern or dot-blots.

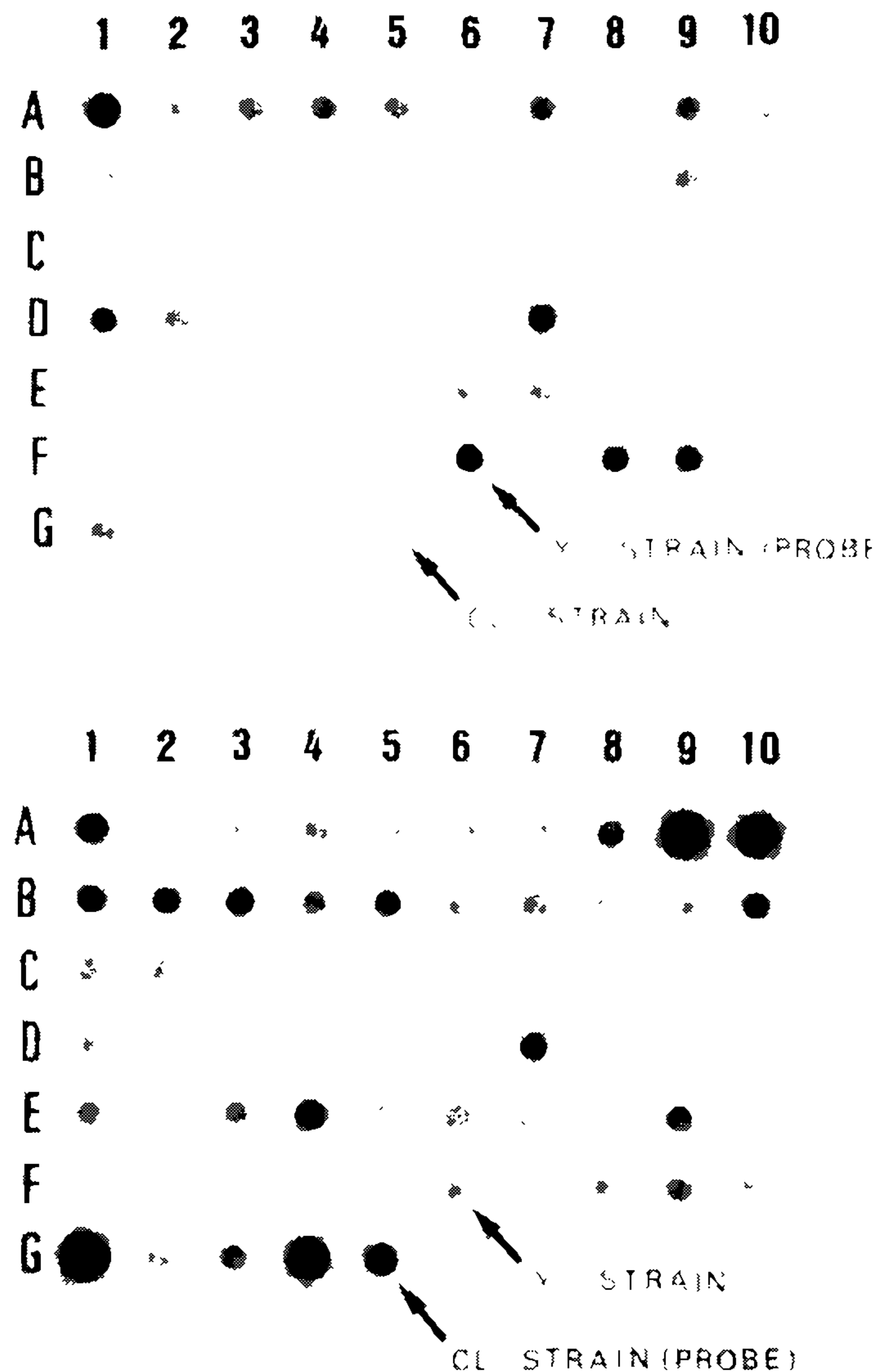

Fig. 1: dot-blot hybridization of kinetoplast DNA isolated from 65 different strains of *T. cruzi* with total kDNA probes from Y and Cl reference strains. 25 nanograms of kDNA fractions prepared from each strain as described by Morel et al., 1980 were applied to nitrocellulose filters with the help of a dot-blot apparatus. Pre-hybridization and hybridization were done in 50% formamide at 37°C using non-fat dry milk as a replacement for Denhardt's solution as described (Johnson et al., 1984). Sample 5G: Cl kDNA control; sample 6F: Y kDNA control; sample 6G: *Herpetomonas samuelpessoai* control (no hybridization).

The above results indicate that DNA probes, either of nuclear or kinetoplast DNA origin, cloned or synthetic, will become increasingly important tools in the study of Chagas' disease. The development of simple protocols for their routine use in parasite detection, quantitation and characterization in biological fluids, tissue sections or whole organs is under way in several laboratories and will represent an important new tool for the investigators in the field.

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