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Weisblum B & de Haseth P L. Quinacrine, a chromosome stain specific for deoxyadenylate-deoxythymidylate-rich regions in DNA. *Proc. Nat. Acad. Sci. USA* 69:629-32, 1972.
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Sequential AT base pairs nonrandomly distributed in chromosomal DNA enhance fluorescence of bound quinacrine, producing characteristic bands in human metaphase preparations that enable precise identification of each chromosome. Fluorescence of quinacrine or quinacrine mustard in the presence of DNA samples of defined composition and conformation provided the clue to the composition of the DNA in the chromosome responsible for the bands. [The *SCI*® *indicates that this paper has been cited in over 235* publications.]

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Our work had its origin in the specific chromosomal staining procedure utilizing quinacrine mustard (QM) devised by T. Caspersson, S. Farber, and their colleagues.[1] The use of QM, proposed to act as an affinity label for G residues in DNA by interaction between the mustard side chain of QM and N-7 of guanine, was successfully applied by Caspersson and his coworkers[2] to identify each human chromosome uniquely by its characteristic banding pattern. Their work stimulated a period of intense growth in the field of human cytogenetics.

When we began our studies in 1971, there were doubts about the chemical rationale of the QM banding method because quinacrine (Q), lacking a reactive mustard side chain, produced a banding pattern indistinguishable from that produced by QM. The affinity label model predicted that QM should bind to DNA in proportion to the content of G+C. To test this prediction, we performed equilibrium dialysis experiments using a series of bacterial DNA samples with increasing G+C content. We measured the concentration of Q by its absorbance and consistently failed to find significant differences between the amount of Q bound by the set of DNA samples that we used, which included poly d(AT), and homopolymers such as poly dA-poly dT and poly dG-poly dC as well.

The moment of truth came when we noticed that samples containing Q plus poly d(AT) that had been left under fluorescent room illumination appeared to glow against the black bench top, whereas the tubes containing Q plus other DNAs with higher G+C content did not. It was a dramatic moment, just like a discovery in the movies, but minus discovery music in the background. This serendipitous observation suggested that we should measure fluorescence rather than absorbance of Q-DNA complexes, and the results of the next several days' work were summarized in our paper.

Contrary to the prevailing wisdom, our studies suggested that Q (or QM), when it produces chromosome bands, acts as a reporter that binds with nearly equal affinity, by intercalation, to all DNAs (rather than as an affinity label that binds preferentially to G); that the fluorescence specificity resides in the acridine ring system (rather than in the mustard side chain); and that the bright fluorescence signal reported by Q is due to sequential AT base pairs (rather than to guanine). Moreover, guanine was shown to quench Q-fluorescence, thereby actively contributing to *reduced* intensity seen in the alternate dark bands. The affinity label model originally proposed required that the tail wag the dog.

Our findings were presented at the Jerusalem Chromosome Conference in June 1972,[3] and, with the help of a quotation from Isaiah (45:3), many remaining skeptics became converts. We utilized the same experimental method to explain the specificity of chromosome fluorescence produced by treatment with Hoechst-33258.[4] Our interpretation of the chemical nature of the Q bands has recently been tested and confirmed by A.T. Sumner,[5] using X-ray microanalysis to measure the amount of Q physically bound to chromosomes. In these studies he showed that the amount of Q bound under banding conditions does not vary along the length of the chromosome.

In relating our biochemical work to cytogenetics we depended on many fruitful discussions with the late H. Jay Barr and his postdoctoral fellow John R. Ellison. Their parallel cytological studies were published separately.[6] Eeva Thermann Patau and her late husband, Klaus Patau, were likewise very supportive. I continue to repay my great debt to them by my annual lecture in Genetics 452 entitled, "How the Chromosome Got Its Stripes." Pieter L. de Haseth is now on the faculty at Case Western Reserve University, where he has made important contributions in the area of bacterial promoters.

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2. **Caspersson T, Zech L, Johansson C & Modest E J.** Identification of human chromosomes by DNA-binding fluorescent reagents. *Chromosoma* 30:215-27, 1970. (Cited 945 times.)
3. **Weisblum B & de Haseth P L.** Nucleotide specificity of the quinacrine staining reaction for chromosomes. *Chromosomes Today* 4:35-51, 1973. (Cited 15 times.)
4. **Weisblum B.** Fluorescent probes of chromosomal DNA: three classes of acridines. *Cold Spring Harbor Symp.* 38:441-9, 1973. (Cited 70 times.)
5. **Sumner A T.** Mechanisms of quinacrine binding and fluorescence in nuclei and chromosomes. *Histochemistry* 84:566-74, 1986.
6. **Ellison J R & Barr H J.** Quinacrine fluorescence of specific chromosome regions: late replication and high A:T content in *Somoaia leonensis*. *Chromosoma* 36:375-90, 1972. (Cited 130 times.)