Am. J. Hum. Genet. 44:441–451, 1989

The William Allan Memorial Award Address: The Background for the Development of the Chromosome Banding Techniques

Torbjörn O. Caspersson

Institute for Tumor Pathology, Karolinska Institute, Stockholm

May I at first express my deeply felt thanks to The American Society of Human Genetics and its president, Dr. David Comings, for the William Allan Memorial Award. It is a very high distinction which I esteem very highly. I owe a debt of gratitude to the award committee and, particularly, to its chairman, Dr. Stanley Gartler. I also thank Dr. Frank Ruddle for his very kind introductory remarks.

I have chosen to speak about the origin and early history of the chromosome identification by "banding" and then, in particular, of the work of a group in our Nobel Institute for Medical Cell Research at the Karolinska Institute in Stockholm. In this connection it is a pleasant duty for me already here to emphasize the central role in these studies of my long-time collaboratress Dr. Lore Zech.

The development around 1970 of the first chromosome banding technique for identification of chromosomes and chromosome regions rather suddenly gave impetus to work on a considerable number of different lines in genetics in its widest sense. Nowadays banding is a routine laboratory method and—I guess—hardly anyone thinks about its rather complex background.

The very first banding procedure—on which, for instance, our present system of chromosome numbering and identification is based—was by no means a chance finding. It was the result of extensive studies of the chemistry of nuclear structures by aid of quantitative high-resolution microphotometric techniques.

One has to go quite far back in order to find the beginnings of optical high-resolution cytochemistry. The direct reason for the building of the very first instrument–UV ultramicrospectrophotometer—in the mid-1930s was a desire to investigate the distribution of nucleic acids in the nuclear structures in the hope of getting some idea about their function in the cell. *Qualitative* cytochemical work on the localization of various substances in the cell had been tried much earlier, but the results were so inconsistent that the whole field of staining with chemical aims had already fallen into disrepute by the mid-1920s. It is perhaps surprising that that situation did not immediately induce efforts to eliminate the main difficulties by developing truly *quantitative* methods, using the microscope as a measuring tool.

It is perhaps not easy to fathom today that as recently in the early 1930s the knowledge of the composition and of the role of nuclear components was still in such a state that claims could be heard that chromosomes consisted only of proteins and lipids—for there are Feulgen-positive lipids. Nothing was known about the function of the nucleic acids. According to some textbooks, a good guess seemed to be that they were waste products of the cell's purine metabolism. Nature's mysterious way of synthesis of proteins appeared to be close to black magic.

On the other hand, the inducement to efforts to get information about nuclear structures grew stronger and stronger during the same period because of the rapid progress of cytogenetics. Let me only mention Cyril Darlington's precocity theory and Hans Bauer's discovery of the curious giant polytene chromosomes in several species of Diptera. Morphological studies of such extremely large chromosomes gave hope that in the future it might be possible to study also the individual gene in loco during its function—at that time a fascinating aspect.

The reason our group in Stockholm chose to build our first quantitative microspectrophotometer primarily for the UV spectral region was the very high absorption of the group of nucleic acids in the middle UV range (around 265 nm). Furthermore, microscope lenses for UV, which were already available, had twice the resolution attainable in the visible region.

UV-absorption spectra were taken of individual meta-

Received December 20, 1988.

Address for correspondence and reprints: T. Caspersson, Emanuel Birkes väg 2. S-144 00 Rönninge, Sweden.

0002-9297/89/4404-0002$02.00

phase chromosomes from many different plants and animals, including man, and even of individual bands and interbands of *Drosophila* salivary-gland chromosomes. The geneticist Jack Schultz from Morgan's group worked with us in Stockholm in those early days, and we could show changes in individual bands related to functional stages.

This was before World War II. Only a few years later, around 1940, the measuring procedures got so advanced that quite detailed spectra could be obtained even from very small nuclear elements and information could be got not only about nucleic acids present but also about proteins and even certain amino acids.

Still more-advanced spectrophotometers were then built, permitting integrating measurement of the total amounts of absorbing substances in a whole cell or in a part of a cell, despite the optical inhomogeneities in the objects.

By far the most interesting result of these studied was the finding that nucleic acids—far from being waste products—play an absolutely crucial role in the cellular protein synthesis. This was published in early 1941 (Caspersson 1941). After the war, when the channels of communications opened up again, we found that Jean Brachet in Belgium had reached the same conclusions.

Another—to us surprising—observation from that time was the omnipresence of RNA in all growing organisms studied (see Caspersson 1950). Only a few findings had previously been reported in the literature. Also, the RNA distribution in the cell was analyzed, and we observed the curious-looking phenomenom of RNA wandering out of the nucleus into the cytoplasm during rapid synthesis of protein—this was later described in the literature as "messenger RNA."

I will now skip over most of the quantitative cytochemical work, done during the subsequent 15–20 years. The techniques were further improved, important additions to the technical resources being procedures for high-resolution, integrating interferometry, and fluorometry (see Caspersson and Kudynowski 1980). Most studies were concerned with the cell metabolism during normal growth. Very little work was conducted at the chromosomal level.

In the 1960s the general interest in tumor growth increased more and more. Also, in our group much work was pursued on that field. Several research workers, most of them using Feulgen microspectrophotometry, found large differences between the DNA patterns in normal cell populations and those in tumor tissues. Generally larger than normal DNA amounts per cell were observed, as were considerable differences between individual cells in the tumor cell populations.

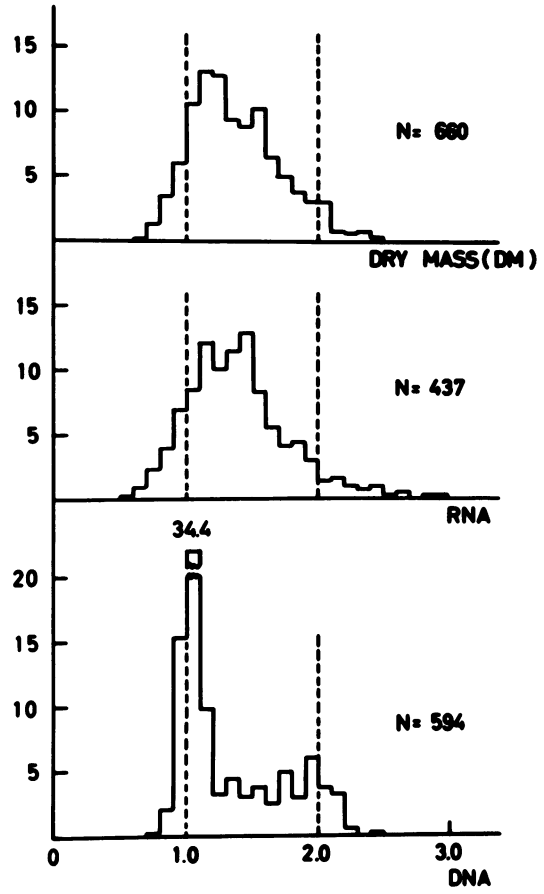


Figure 1 Histograms, in arbitrary units, of the distribution of DNA, RNA, and cellular dry mass, respectively, in cell populations from a normal, growing fibroblast tissue culture. N = no. of cells measured. Dashed lines mark the diploid and the tetraploid DNA values.

Figure 1 shows the DNA histogram (DNA pattern) of a normal growing tissue culture. It is based on spectrophotometric measurements of a couple of hundred individual cells. The distribution patterns of RNA and of the dry weight of individual cells are also given. The DNA histogram has two peaks. The left one represents the "resting" cells, which have recently divided. The right peak represents the tetraploid cells, which have finished the premitotic DNA synthesis and are ready to divide. The region between the peaks contains cells in different stages of DNA synthesis. In nongrowing ("resting") tissues, all or almost all cells lie in the area of the left, diploid peak. As soon as the population starts to grow, the second peak appears.

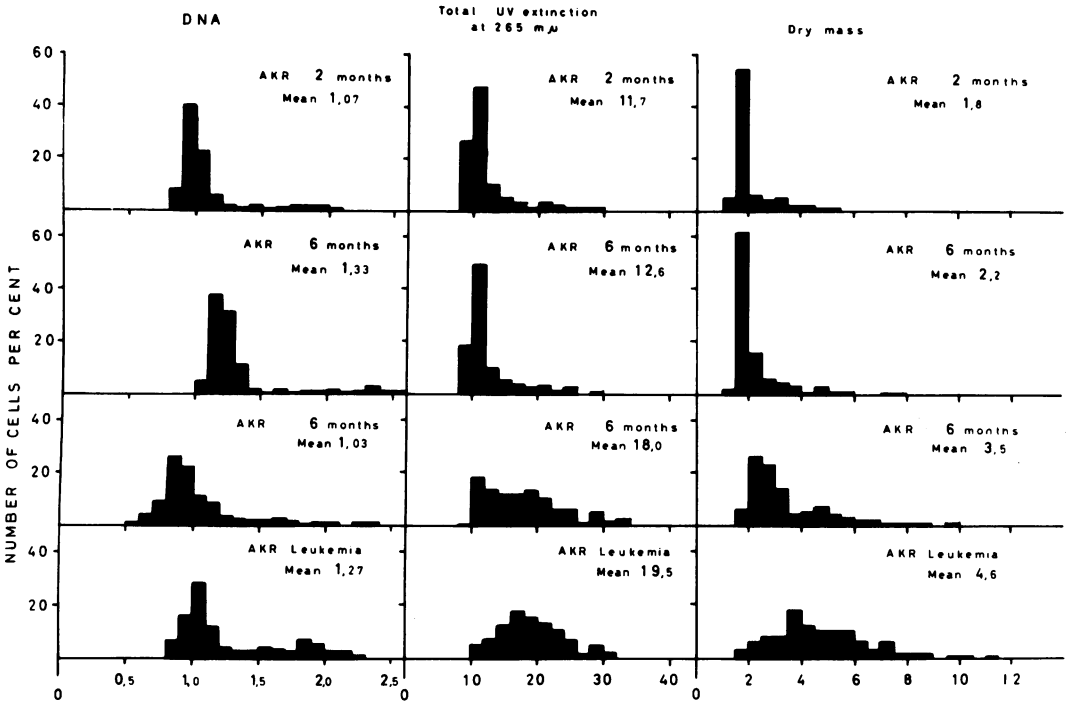


Figure 2 Distribution diagrams of DNA, DNA+RNA (measured by UV absorption), and cellular dry weights from thymus cells from AKR mice. The top now shows data on a nonleukemic mouse; the bottom row shows data from an old mouse with established leukemia. The two middle rows show data from the period of the onset and come, respectively, from a mouse that has developed leukemia and from one that has not yet reached that stage.

For the discussion that is to follow, it is important to remember than in normal cell populations there are no cells present with DNA values outside the range depicted in the figure 1.

Lymphoid cells offer especially good opportunities for precise cytochemical work. Figure 2 (from Gahrton et al. 1966) shows distribution patterns of DNA, DNA + RNA, and cellular dry weight of populations of thymus cells from leukemic mice of the AKR strain. This strain has an incidence of 89% for spontaneous leukemia, which sets in between the ages of 6 and 12 mo. In the figure the upper set of histograms comes from an as yet "normal" nonleukemic mouse, and the set at the bottom comes from an old mouse with established leukemia. The two sets of diagrams in the middle are both from the period of the onset and come, respectively, from a mouse that has developed leukemia and a mouse that has not yet reached that stage.

Similar findings, a broad DNA-distribution pattern with many cells lying outside the normal DNA ranges, have been made by many authors in many types of tumor. In spite of that, the whole body of DNA literature presents a quite confusing picture. Most of the studies mentioned were aimed at using DNA determination as an aid to cytodiagnostics, but success was very limited in that it was soon found that such measurements yielded little more information than did cytomorphology. What has so often been overlooked or disregarded is the fact that there are many tumors in which no DNA deviations from normal can be found—they are either too small or nonexistent. Recent work on human tumors of different kinds has shown that this situation is far from uncommon. Figure 3 gives some examples from a series of malignant cervix carcinomas (Caspersson 1964). In two of the cases the DNA patterns are well inside the limits of the DNA distribution of normal cell populations.

With very great probability we must assume that in every abnormally growing tumor cell there are deviations from normal in parts of the gene-carrying chromosomal DNA. This applies also to the tumor cases in which no changes from the normal DNA amount can be demonstrated with our chemical measuring methods. This means that, in order to penetrate deeper

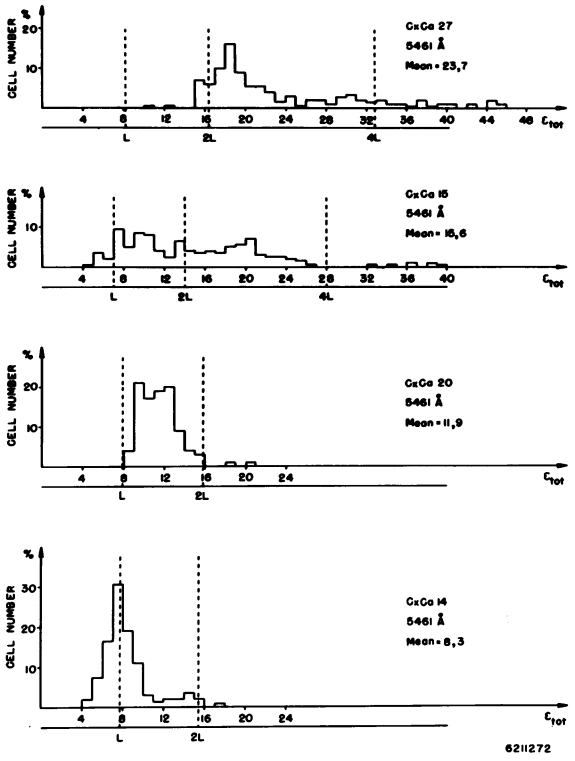


Figure 3 DNA histograms from cervical scrapings from four patients with uterine cervix carcinoma.

into the mechanisms of carcinogenesis, it is necessary to try to obtain chemical and/or structural information from individual chromosomes, by looking for aberrations or other changes in the parts of the genome an individual chromosome carries.

The reaction of our research group to this situation at the end of the 1960s was at first to try to find out what our type of quantitative cytochemistry could offer for studies of chromosome details.

Already, at the very beginnings, the way appeared to be blocked by the deplorable state of chromosome identification in man at that time. Without an absolutely reliable method to recognize every individual chromosome, any effort toward a detailed chromosome analysis would be meaningless.

The state of the methods for chromosome identification was at that time indeed primitive. The only really reliable characteristic, useful for chromosome identification, was chromosome length. On the basis of that, the human karyotype was generally described as consisting of seven chromosome groups, labeled A–O. Within each group the length differences were so small that it was not possible to distinguish between the individual chromosome types, except for the A group, containing the three longest chromosomes types (numbers 1–3). The other groups were as follows: group B, two chromosome types; group C, eight chromosome types; group D, three chromosome types; group E, three chromosome types; group F, two chromosome types; and group G, containing the three very smallest chromosome types. That means that, of the karyotype's 24 different chromosome types, only three could be identified with certainty. Some authors claimed that, using other, lesser, morphological features, one could, in addition, identify three or possibly four other types. Even if that is accepted, there still remained 17 or 18 unidentifiable chromosome types. Thus, the first step in our research project would have to be development of a reliable chromosome identification procedure.

We tried hard to bring our main optical cytochemical tools for high-resolution work (micro-interferometry and high-resolution fluorometry) to work well also with the very small chromosomes one finds in many species, including man.

Using the extreme resolution of the UV-microscope optics, we could in some cases find reproducible DNA-distribution patterns along a metaphase chromosome. Figure 4 shows an example (Heneen and Caspersson 1971). The test object is the plant rye, which has only seven chromosome pairs. Their length is comparable with that of the largest human chromosomes. In con-

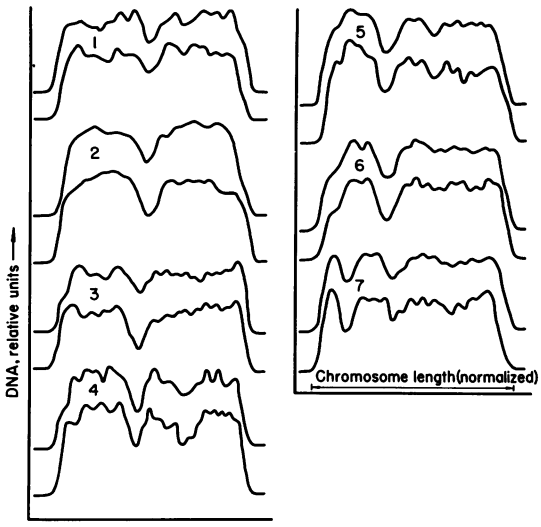


Figure 4 DNA distribution along individual chromosomes from rye, measured by high-resolution, scanning UV spectrophotometry. The seven chromosome types can be recognized by their DNA patterns.

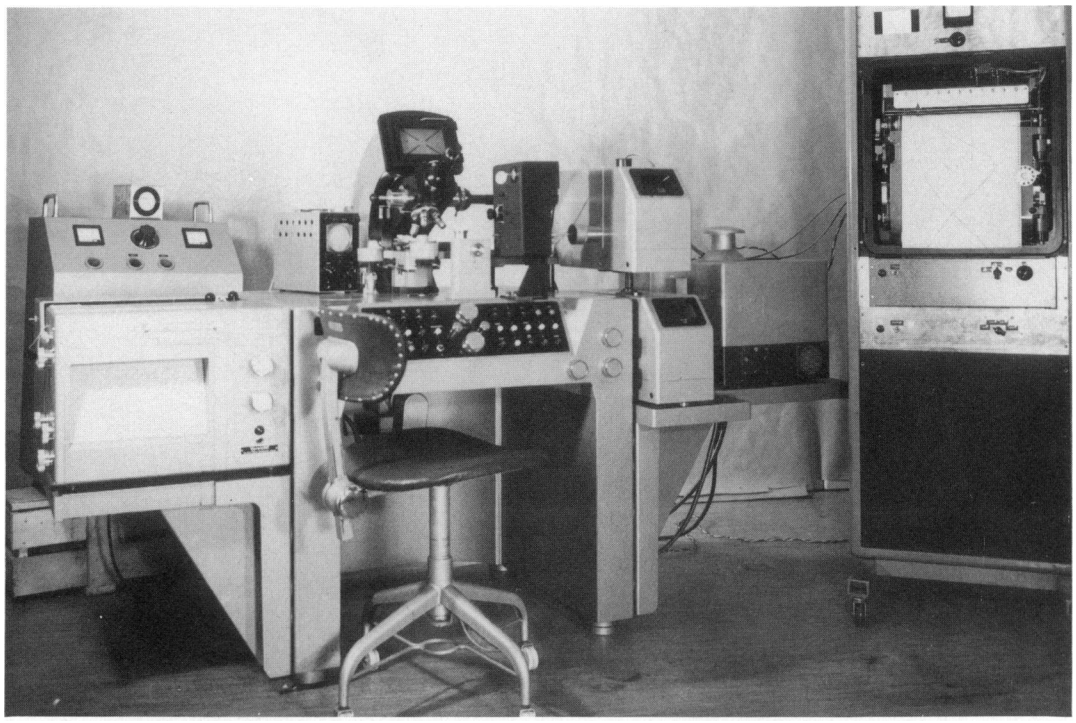


Figure 5 Recording, scanning high-resolution microspectrophotometer for UV and visible spectral regions (230–700 nm)

ventionally stained preparations the seven chromosomes—being of approximately the same length—resemble each other to such an extent that it is not possible to distinguish between them with any degree of certainty. In the UV microscope, used at the nucleic acid absorption maximum (265 nm), faint irregularities in the absorption pattern along the chromosomes could be discerned. They were, however, far too indistinct to permit reliable identification. For physiological reasons, recording measurements give more precise information about such types of patterns than the eye can perceive.

By running a transverse, narrow slit along a chromosome in the UV microspectrophotometer, continually recording the absorption (or absorbance), one can get a curve describing the nucleic acid distribution along the chromosome. The "width" of the slit must be somewhat below the resolution limit of the lens used. These measurements were carried through in a recording microspectrophotometer, constructed especially for work on very small structures. It was built as a joint project between our laboratory and the West German company Zeiss (fig. 5).

The recordings of the rye chromosomes demonstrated, in every metaphase studied, seven types of DNA-distribution patterns, which with reasonable probability could be assigned to the seven different chromosome types.

This looked, to begin with, quite promising for our identification project. However, when this technique was applied to the human karyotype, entirely satisfactory recordings could only be obtained from the five longest chromosomes (groups A and B). The complex C group with its eight members could not be broken up in a reliable way, and the still smaller chromosomes presented still greater difficulties. Even in the cases where differences between the DNA-distribution patterns made it possible to distinguish between chromosome types, the patterns showed few details (see fig. 6, upper curve).

In the hope of getting more chemical information than nucleic acid determinations can give, Dr. Lore Zech and I tried the following approach: It is well known that the genes are linearly arranged along the chromosome and, furthermore, that the gene specificity is carried by the pattern of distribution along the gene of the purine and pyrimidine bases. Then it is to be expected that genes with different functions should differ in content and also in internal distribution of individual bases. For strictly statistical reasons it appears proba-

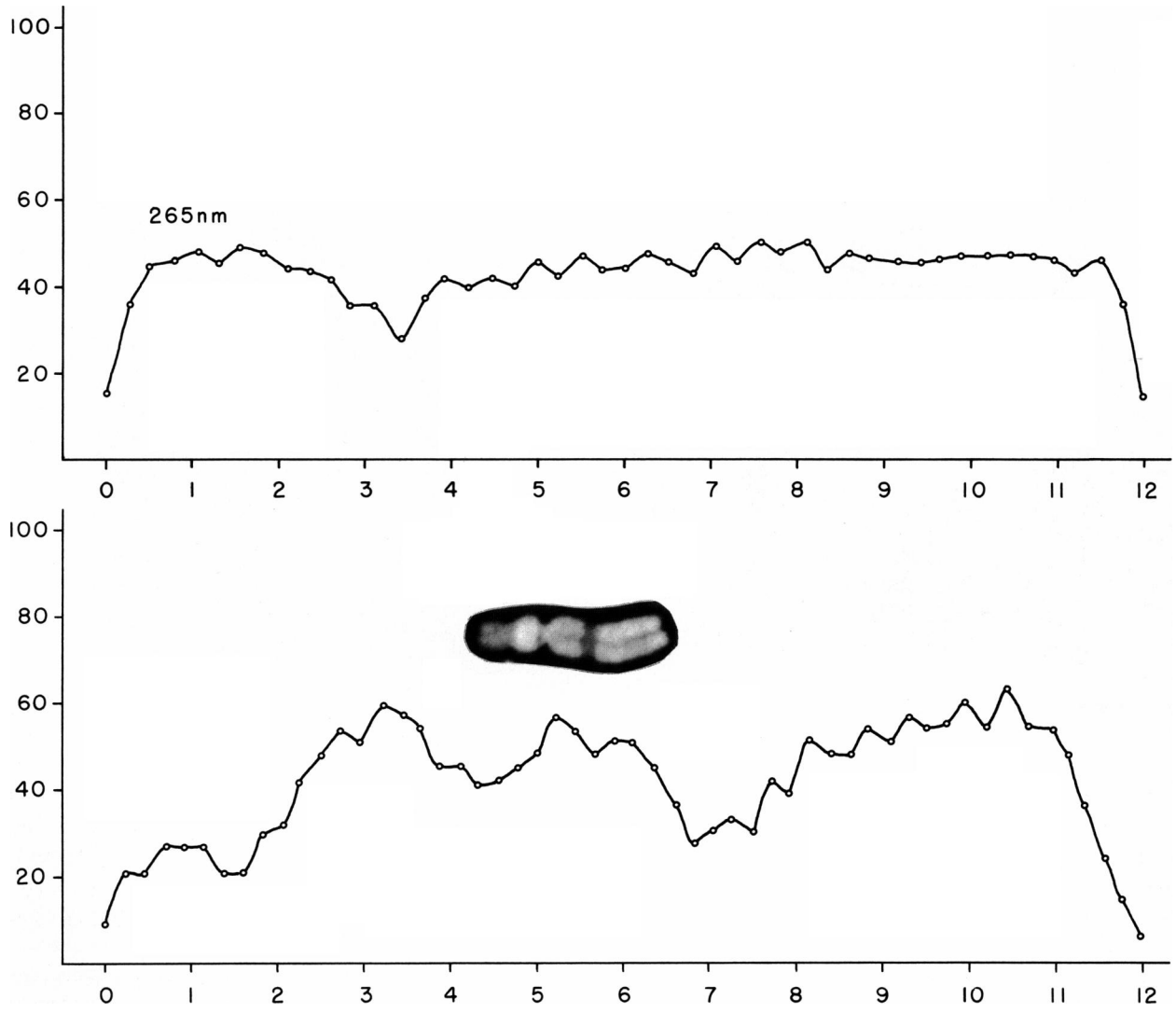


Figure 6 Scanning measurements on a chromosome from Scilla sibirica. Chromosome length is 12 microns. *Upper curve,* DNA distribution measured at the nucleic acid absorption maximum. *Lower curve,* Distribution of fluorescence after quinacrine mustard staining.

ble, then, that different regions in one and the same chromosome should contain different proportions of the individual bases. If one in some way could mark one or more of the bases—or, possibly, certain base sequences—that should result in some kind of pattern along the chromosome, which could be expected to carry more information than does the simple DNA-distribution pattern.

To make a long story short: we tried a number of base-binding compounds, preferably those with strong fluorescence. For reasons I cannot go into now, fluorescence microscopy can, in very careful work, permit work with very high optical resolution.

We got the best results with the acridine quinacrine mustard, a compound with high fluorescence which was known to react with amino groups—guanine has a free amino group. We know nowadays that the mechanism of fluorescence banding by aid of quinacrine mustard is somewhat more complex than simple amino group binding, but that changes nothing in principle.

Figure 6 shows, at the top, the DNA-distribution pattern of a rather large (12-micron) plant chromosome (Scilla sibirica) and, below, the fluorescence pattern about quinacrine staining. The latter pattern shows much more detail than the DNA pattern—as was to be expected.

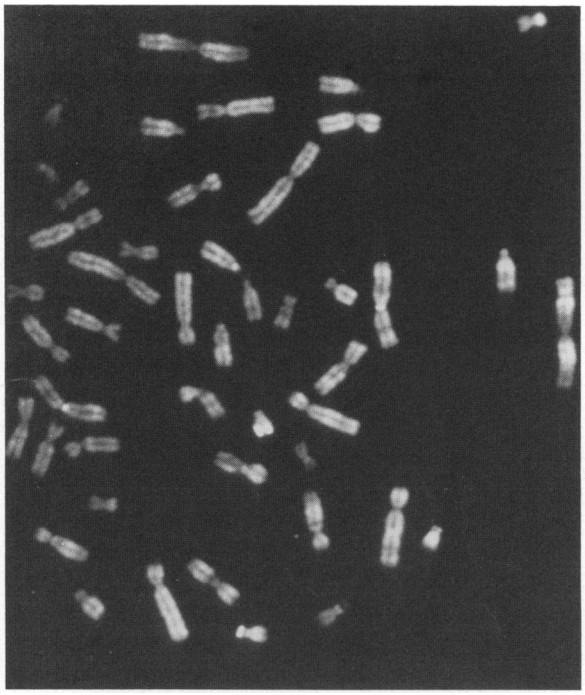


Figure 7 Quinacrine mustard–treated human metaphase. All chromosome types are visible.

In quinacrine mustard–stained human metaphases, all chromosomes appeared to be in some way structured (fig. 7). This looked promising, but, if one hopes to base a chromosome identification system on these fluorescence patterns, these patterns would have to be proved reproducible and different for all the 24 human chromosome types. In order to test or prove that, it would obviously be necessary to collect a large enough number of observations to allow statiscal analysis. To do this by visual observations in the microscope would be an almost impossible undertaking. The only way out, then, is to introduce some kind of objective measuring procedure (see Caspersson et al. 1969, 1970).

The pattern of fluorescence along an individual chromosome can be measured and recorded in a high-resolution fluorometer. We found with such an instrument (Caspersson et al. 1980) so many different fluorescence patterns that it appeared probable that they were different for all of the different chromosome types. For a large-scale study that instrument was far too slow, and we built a recording instrument, working with photographic photometry (fig. 8*A*, 8*B*), which permitted very much faster recordings. The accuracy was somewhat lower than that for the first-mentioned instrument, but this did not impair the judgment of the shape of the fluorescence curves.

Again I will make a long story short: to begin with we recorded the fluorescence patterns or "fluorescence profiles" along individual chromosomes from a series of human metaphase plates—altogether, about 5,000 chromosomes were measured. The profiles could rather easily be grouped into 24 categories. Figure 9 demonstrates the different types of profiles, with the most conspicuous band marked by vertical lines. Recordings were thereafter made of close to 20,000 additional chromosomes, and the groups proved to be entirely reproducible.

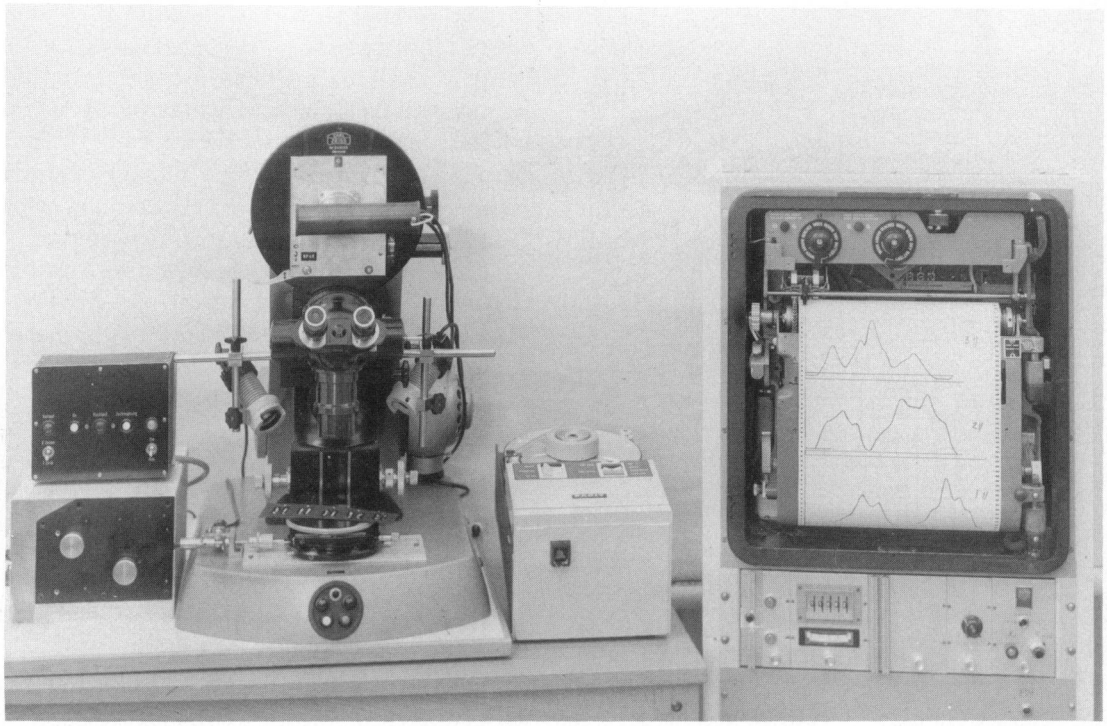
In Caspersson et al. (1971*a*, 1971*b*, 1972), among other things statistical analysis of the material and also certain procedures to get more details in the profile measurements are described. In these publications measurements of the meiotic chromosomes are described, as are studies of the variability of certain patterns. Examples are given of analysis of different types of chromosome aberrations.

When the profiles proved so reproducible in the large series of measurements, we thought the time was ripe to christen the more than 16 chromosome types that had previously been unclassifiable. When feasible, the conventional principle of numbering the types according to length was followed—the length differences within the individual groups are, however, so small that the principle is rarely of any practical importance. (Caspersson et al. 1969, 1970, 1971*b*). The numbers assigned can be seen in figures 9–11, the latter of which shows the "fluorescence karyotype" of man.

At the Paris Conference on standardization in Human Cytogenetics in 1971, our numbering system was officially accepted, and work was begun also to develop nomenclature for individual chromosome regions. This latter—as all of you know—has been a very great help in the analysis of aberrations and in the work on human gene mapping.

As was said in the beginning of this presentation, the immediate reason for our efforts to build up a system for chromosome identification was a desire to be able to look for and to analyze chromosome aberrations in tumors. We immediately began work of that kind. Very soon, other groups started on that field, which expanded fast. I do not have the time to go into details here.

Quite soon after the publication of the fluorescence banding technique, other banding techniques were worked out in other laboratories. The majority were based on modifications of Giemsa staining. Certain of



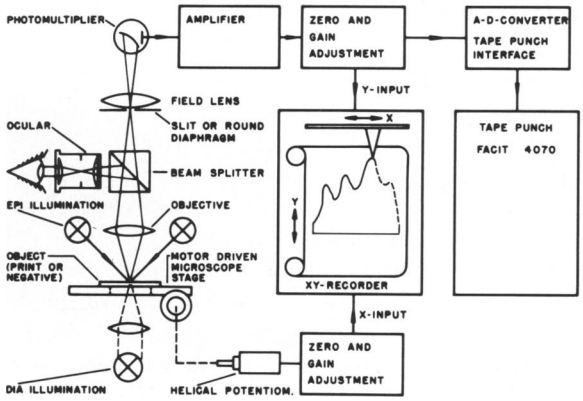


Figure 8 *A*, Photographic fluorescence measuring photometer used for large-scale fluorescence-profile measurements. *B*, Diagram of fluorometer in fig. 8*A*.

them are, for routine work, much more convenient and faster than the somewhat cumbersome fluorescence analysis. It should, however, be remembered that the methods give somewhat different kinds of information and that the methods supplement each other.

The only reason for the photoelectric recording, as described above, was the need to collect such a large number of objective profile measurements that it could be statistically analyzed. In the present day, routine work recordings would hardly ever be necessary. The cytochemical measuring devices, however, served their purpose very well. Without them, I doubt that it would have been possible—in any case, in a reasonable time—to get together such a very large amount of material for observation that it could give a solid basis for a reliable identification system.

It was rather soon possible, by improvements in fluorescence banding as well as in other banding techniques, to get much more detailed information about the chromosomes structure (Caspersson et al. 1972).

That is, however, outside the scope of this presentation. You are all also well aware of the very many applications of banding techniques, nowadays, in medicine and general biology, and I will stop here, as my task was to speak about the very beginnings only.

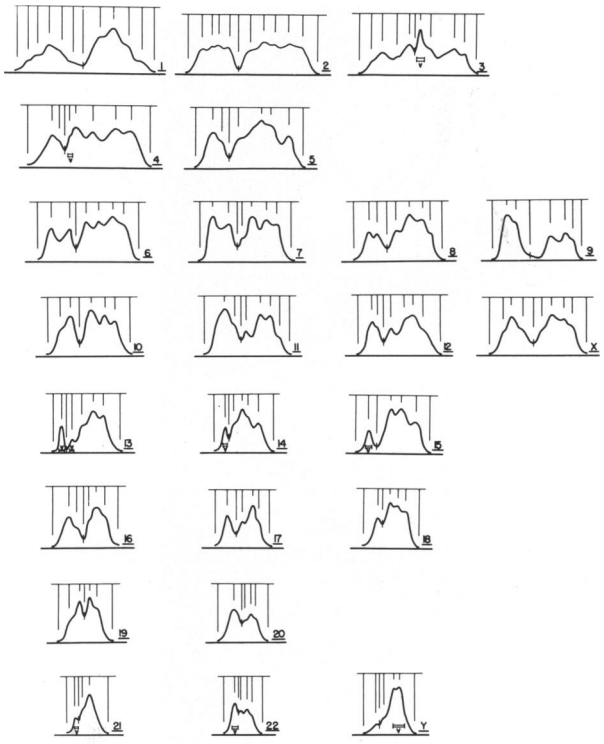


Figure 9 Twenty-four different and characteristic fluorescence profiles of the 24 human chromosome types. The most conspisuous bands are marked by vertical lines.

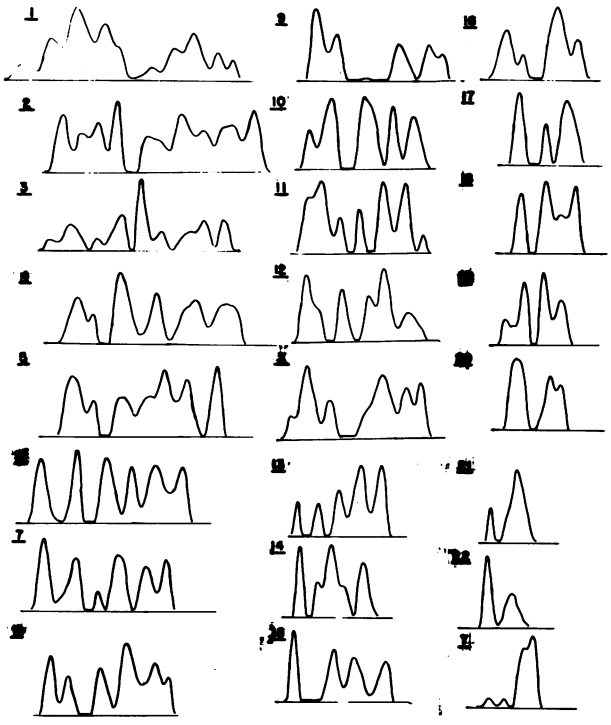


Figure 10 The contrasts in the fluorescence patterns can be artificially enhanced by optical and/or electronic means. This is most easily done electronically, as in the figure above. Such procedures can simplify analytic banding work as well by fluorescence as by Giemsa techniques.

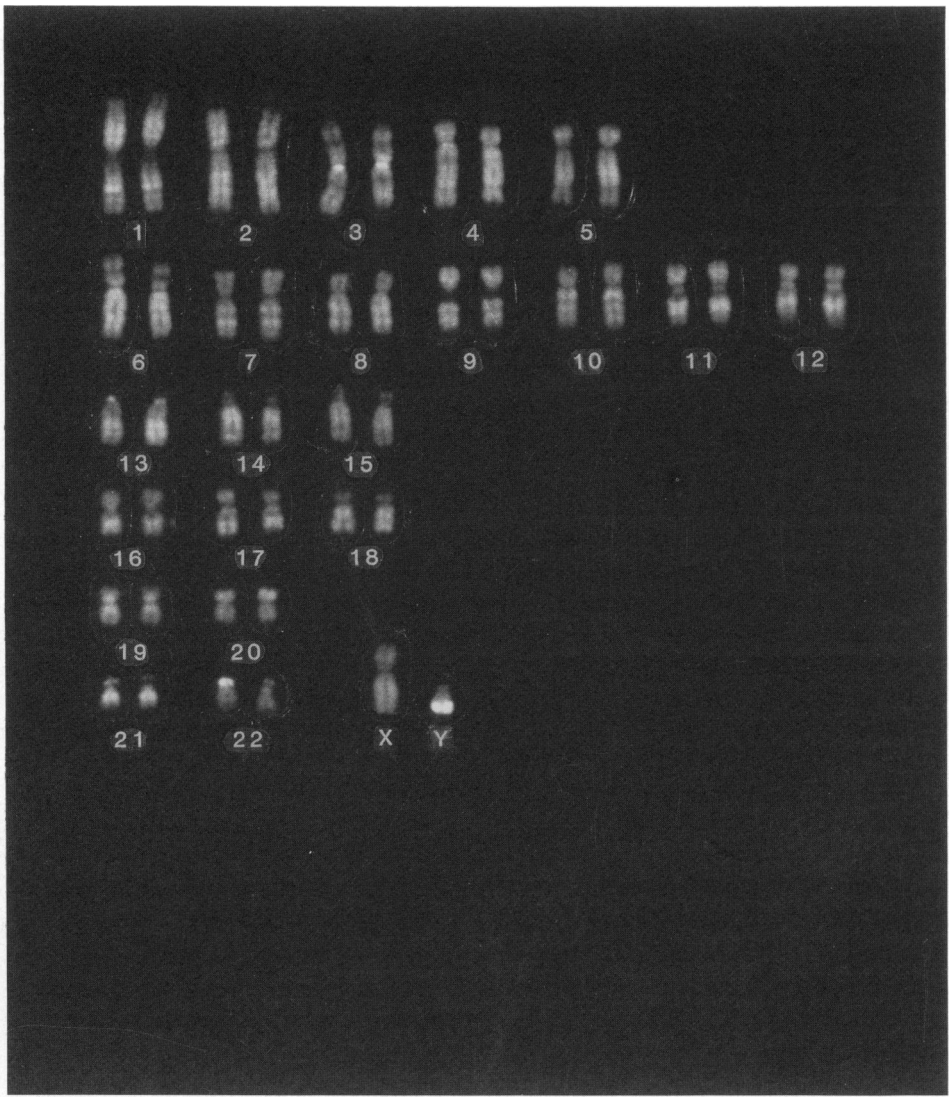


Figure 11 The human karyotype, quinacrine mustard fluorochroming.

References

Caspersson, O. 1964. Quantitative cytochemical studies on normal, malignant, premalignant and atypical cell populations from the human uterine cervix. Acta Cytol. **8**:45–60.

Caspersson, T. 1941. Studien ueber den Eiweissumsatz der Zelle. Naturwissenschaften **20**:33–43.

———. 1950. Cell growth and cell function. W. W. Norton, New York.

Caspersson, T., and J. Kudynowski. 1980. Cytochemical instrumentation for cytopathological work. Int. Rev. Exp. Pathol. **20**:1–54.

Caspersson, T., J. Lindsten, G. Lomakka, A. Möller, and L. Zech. 1972. The use of fluorescence techniques for the classification of mammalian chromosomes. Int. Rev. Exptl. Pthology Vol. **11**:1–72.

Caspersson, T., G. Lomakka, and A. Möller. 1971*a*,. Computerized chromosome identification by aid of the quinacrine mustard fluorescence technique. Hereditas **67**:103–110.

Caspersson, T., G. Lomakka, and L. Zech. 1971*b*. The 24 fluorescence patterns of the human metaphase chromosomes—distinguishing characters and variability. Hereditas **67**:89–102.

Caspersson, T., and L. Zech. 1972. Fluorescent labeling and identification of human chromosomes. Pp. 163–185 *in* S. W. Wright, B. F. Crandall, and L. Boyer, eds. Perspectives in cytogenetics: the next decade. Charles C. Thomas, Springfield, IL.

Caspersson, T., L. Zech, and C. Johansson. 1970. Analysis of the human metaphase chromosome set by aid of DNA-binding fluorescent agents. Exp. Cell Res. **62**:490–492.

Caspersson, T., L. Zech, C. Johansson, and E. J. Modest. 1970. Identification of human chromosomes by DNA-binding fluorescent reagents. Chromosoma **30**:215–227.

Caspersson, T., L. Zech, E. J. Modest, G. E. Foley, U. Wagh, and E. Simonsson. 1969. DNA-binding fluorochromes for the study of the organization of the metaphase nucleus. Exp. Cell Res. **58**:141–152.

Gahrton, G., W. Habicht, and B. Wahren. 1966. A quantitative cytochemical investigtion of DNA, RNA and protein in lymphoid cells from normal, preleukemic and leukemic mice. Exp. Cell Res. **42**:218–229.

Heneen, W. K., and T. Caspersson. 1971. Identification of the chromosomes of rye by distribution patterns of DNA. Hereditas **76**:259–272.