

## Milestones in Chromosome Preparation

- **1955.**

Until the mid 50s', it was commonly thought that the number of human chromosomes was 48.

The 22nd of December 1955, a Chinese scientist called Tjio, occasionally hosted by the Laboratory of Genetics at the University of Lund (Denmark) directed by Professor Levan, was observing "cultures of human embryonic lung fibroblasts, grown in bovine amniotic fluid" , that were showing an high number of cells in mitosis. Chromosome preparations were made by Tjio according to the protocol suggested by Hsu (an American scientist working at the University of Galveston, Texas - USA), slightly modified, i.e. after the treatment with colchicine (12-20 hours) to stop the cell cycle, the cells were exposed to hypotonic solution but reducing the total time to only 1-2 minutes. He then proceeded to "fixation in 60% acetic acid *twice exchanged in order to wash out the salts left from the culture medium and from the hypotonic solution...*".

Finally he applied the "squashing" technique, followed by orcein staining.

To his own big surprise, he could observe chromosomes much better spread and bigger compared to the ones obtained by Hsu (see figures 1 and 2).

Figure 1: Typical metaphase obtained by Hsu

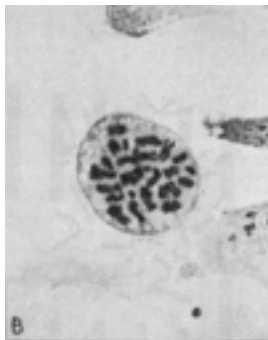


Figure 2: Typical metaphase obtained by Tjio



On top of that, when Tjio and his colleagues carefully counted the chromosomes, in 261 metaphases out of 265 the number was 46 and not 48 as universally taught and claimed by Hsu himself.

This discovery, considered to represent the starting point of the human cytogenetics, was the subject of a scientific paper published in January 1956. <sup>(1)</sup>

Although absolutely dictated by casualty, the introduction of acetic acid in aqueous solution was the principal reason of those unexpected results, and, as a matter of fact, since then the acetic acid will never leave the stage.

According to the recent scientific findings, the acetic acid in aqueous environment is the essential condition to obtain those phenomena of spreading and swelling of chromosomes, which are fundamental for the chromosome analysis.

- **1959.**

Jerome Lejune *et al.* noticed the presence of a small extra-chromosome in all mitosis obtained from cultures of fibroblasts belonging to nine individuals affected by Down's syndrome (trisomy of chromosome 21) <sup>(2)</sup>.

In the same year, Patricia Jacobs observed the presence of an extra-X chromosome in male patients suffering from the Klinefelter's syndrome (47, XXY); cultures, in this case, were set up from bone marrow cells obtained by sternal puncture. <sup>(3)</sup>

Based on these results, the researchers became more and more oriented towards the clinical application of Cytogenetics.

However, the techniques used did not become popular due to both the nature of samples used (solid tissues) and the high variability of the quality of chromosomes obtained.

- **1960 - 1970**

While attempting to find cellular substrates that might help reducing the variability of results, and at the same time making easier the access to the samples, Moorhead started to use PHA (phytohaemagglutinin) stimulated lymphocytes. <sup>(4)</sup>

A significant improvement introduced by Moorhead in the chromosome preparations procedure was a new kind of fixative, now formulated as a mixture of methanol and acetic acid (3:1). Thanks to this new formulation, (along with the air drying of the slides, which can also be quickly passed on a flame), it was possible to replace the aqueous acetic acid and the squashing technique previously used in the final step of the chromosome preparations.

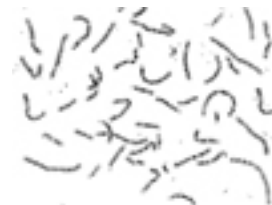
These improvements proved to be so significant that this technique is still used nowadays in cytogenetic laboratories.

In these years Caspersson (QFQ)<sup>(5)</sup>, and Seabright (GTG)<sup>(6)</sup> introduced the banding technique, allowing a real analysis of the chromosome structure as well as an unambiguous pairing of chromosomes' couples (see figures 3-4).

Figure 3: Example of QFQ banding



Figure 4: Example of GTC banding

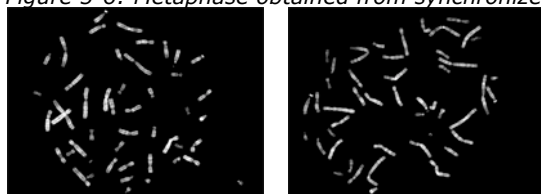


These works encouraged the development of other staining techniques, such as RBA, Da-DAPI, Ag-NOR.

Yunis *et al.* then designed the high resolution banding technique <sup>(7)</sup>, made possible by cell cycle synchronization

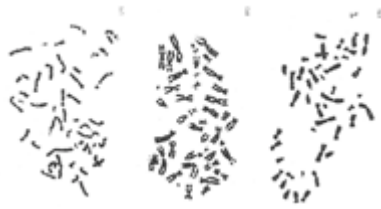
Concerning this, it is particularly noticeable the paper of Webber and Garson "Fluorodeoxyuridine Synchronization of Bone Marrow Cultures", 1983) <sup>(8)</sup>. This new synchronization technique was very efficient and simple, to the extent that many laboratories started to use it routinely for lymphocyte cultures, too (see figure 5-6).

Figure 5-6: Metaphase obtained from synchronized culture of lymphocytes



Later on (1987), Gibas will adopt this approach to synchronize cytotrophoblast cells from CVS (Chorionic Villi Samples)( see figure 7).

Figure 7: Examples of Metaphase obtained from CVS using FdU synchronization method.



- **1960 – 1970**

At the end of the 60' it was discovered that cells of fetal origin, that can be found in amniotic fluid samples, showed the ability to form clones producing a number of cells in mitosis <sup>(9)</sup>.

The steps of the chromosome preparation (colchicine, hypotonic treatment, fixation in methanol-acetic acid 3:1, air drying, differential staining) were essentially the same as those routinely embraced for the chromosome preparations from PHA stimulated lymphocytes.

This was indeed the start of the pre-natal diagnosis.

- **1980**

At the beginning of the decade a new semisynthetic culture medium formulated by Chang became available: this medium enabled the laboratories to reduce considerably the time of culture (from 20-30 to 8-12 days) and to significantly increase the number of clones. <sup>(10)</sup>

- **1983**

On February 3<sup>rd</sup> in the Cytogenetic Laboratory of the Ist Clinica Ostetrico-Ginecologica "Mangiagalli" (University of Milan), a trisomy 21 was diagnosed from cells in spontaneous mitosis obtained from a sample of Chorionic Villi ( CVS) taken during the first trimester of pregnancy <sup>(11)</sup>. Since then, a new material such as the Chorionic Villi (allowing early diagnosis, i.e. during the first trimester of pregnancy) was made available.

Moreover a new, or rather a different technique to obtain analyzable chromosomes, was introduced. Interestingly, the cytotrophoblast cells in spontaneous mitosis were obtained using 60-70% aqueous acetic acid <sup>(12)</sup>.

Although this "direct technique" allowed a quicker diagnosis, yet the quality of the chromosomes was not satisfactory.

This is the reason why long term cultures of mesenchymal cells took off, as they made possible the chromosome banding with a quality level comparable to the one achieved with cultures of cells from amniotic fluid. <sup>(13)</sup>.

As mentioned before, was only in 1987 that Gibas *et al.* succeeded to synchronize the cytotrophoblast cells, hence he could obtain a good quality banding with the direct technique, too ("A simple technique for obtaining high chromosome preparation from chorionic villus samples using FdU synchronization") <sup>(14)</sup>.

- **1985**

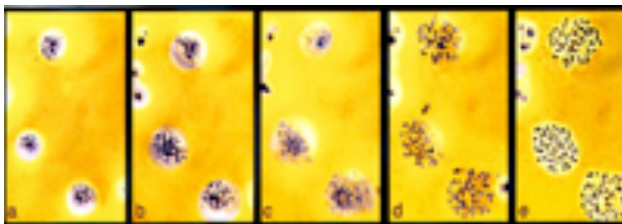
In a "Letter to the Editor", published in "Prenatal Diagnosis", Lundsteen <sup>(15)</sup> reported the results of his experiments dealing with the role of temperature and relative humidity. It was clear from his findings that these two parameters do have a significant impact on

the chromosome preparations. From a technical standpoint, this translated in a very important concept, namely that by properly controlling temperature and relative humidity, one can expect that the quality of chromosome preparations becomes consistently good – regardless of seasonal changes and environmental factors.

- **1988**

At the XXI Symposium on Cytogenetics, held in Praga in August of that year, a sequence of pictures (shot during the final evaporation of the fixative) was presented by G. Terzoli. For the first time it was shown in a very evident way that the spreading of chromosomes does occur during that phase; at the same time, the chromosomes dimensions expand in a considerable and unexpected way (see figure 8).

Figure 8: Sequence showing how the spreading of chromosome occurs during the final evaporation of fixative (aqueous acetic acid).



- **1990**

From the end of this decade, the image analysis computerized systems started to be more and more widely used in the cytogenetics labs. This technical advance substantially contributed to improve the resolution of chromosome banding, offering as well the possibility to perform karyograms quicker and easier, so that the whole chromosome analysis became much more reliable.

In an article published in 1996 Spurbeck et al. <sup>(16)</sup> demonstrated that during the final evaporation of the fixative the chromosome spreading increases as the environmental relative humidity increases.

- **2002**

*"Demystifying chromosome preparation..."*: this work, written by Claussen <sup>(17)</sup>, although not so well known, resulted in a real milestone among the studies regarding the chromosome preparation steps.

This article, published in "Cytogenetic and Genome Research", is so important that deserves a summary of the key points:

- 1) During the hypotonic treatment the chromosomes move from the center of mitotic cells (which are spherically shaped) towards the cell's membrane
- 2) When the fixative reaches the cells, the outer and inner membranes are disrupted
- 3) In presence of water and acetic acid, both the cytoplasmatic and the chromosomal proteins swell, clearly and independently
- 4) When the final evaporation of fixative occurs in an environment without water, the swelling of the cytoplasmatic proteins is inhibited and the same happens to the swelling of the chromosomes, so that there is no increase in their dimensions
- 5) When the ambient humidity increases, the spreading and the sizes of the chromosomes increase, too.

The conclusion was that the swelling of cytoplasmatic and chromosomal proteins in mitotic cells during evaporation of the fixative is, in reality, *'based on an interaction*

*between acetic acid, water and cell proteins*'. Definitely a giant move towards the end of "magics" in cytogenetic laboratories, and a great contribution to the beginning of "science"!

- **2006**

On occasion of the IX Congress of the Italian Society of Human Genetics (SIGU), held in Venezia-Lido 8-10 November, following the speech of Dr. Claussen dealing with the topic mentioned above, G. Terzoli showed and commented a short video filmed at the microscope.

For the first time the cytogenetists attending this presentation could actually see what happens to the mitosis during each step of the chromosome preparation. It was finally clear in front of them the behavior of cells and chromosomes during the hypotonic treatment, in the fixative solution and (mostly important) during the final evaporation of the fixative in different conditions of relative humidity.

During the same presentation, it was proposed that some mechanism involving protein hydration could be the cause of the swelling of both cytoplasmatic and chromosome proteins. Based on this hypothesis, the hydration produces the swelling of the cytoplasmatic proteins, that in turn gives raise to the chromosome spreading. In the case of the chromosomes, the same hydration process causes the swelling of the proteins that increases the three-dimension size of the chromosomes; consequently, more or less condensed areas along the chromosome itself (that after staining will be seen as bands) are originated.



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