Series
DOI 10.1007/s12038-014-9417-0

What history tells us XXXIII. Molecular hybridization: A problematic tool for the study of differentiation and development (1960–1980)

Michel Morange

Centre Cavaillès, République des Savoirs USR 3608, Ecole Normale Supérieure, 29 Rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, 33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

Molecular hybridization consists of the formation of double-stranded DNA-DNA or DNA-RNA (less frequently RNA-RNA) duplexes between single-stranded polynucleotides with complementary sequences. It is one of the techniques that has been most extensively used in molecular biology. DNA sequencing and amplification of DNA by PCR are based on it. The same is true for the Northern and Southern blot experiments, extensively used by molecular biologists during the early days of genetic engineering, before PCR and the advances of the sequencing programmes. *In situ* hybridization has been and still is a highly used technique to map genes on chromosomes, and to study gene expression – both its level and cellular localization – during development.

But there was another dimension to molecular hybridization that occupied a major place in the 1960s and 1970s, and completely disappeared at the beginning of the 1980s: the use of this technology to access the structural organization of the genome and to investigate the way the genetic information contained in the genome is expressed during differentiation and development. It was a global approach, ignoring the precise nature of the DNA and RNA sequences. This contribution is devoted to this use of molecular hybridization and to the difficulties encountered.

2. Origin and development of the work

This work has its origins in the study and interpretation of DNA denaturation, initially done by René Thomas and then developed at Harvard by Paul Doty and Julius Marmur (Giacomoni 1993). The first hybrid between DNA and RNA was obtained in 1960 by Alexander Rich (Rich 1960). Doty and Marmur showed in the same year that it was possible to renature DNA by pairing its separated strands, and to recover a fully functional DNA molecule – in its capacity to transform bacteria (Marmur and Lane 1960; Doty *et al.* 1960).

The merit of having recognized the potential applications of this method has to go to Sol Spiegelman. He had not previously been successful with his hypothesis on the existence of plasmagenes (Spiegelman and Kamen 1946) or in the long controversy he had with Jacques Monod on enzymatic adaptation. Yet, Spiegelman now saw that the new method could be used to check the hypothesis of messenger RNA. While in 1961 the existence of rapidly labelled RNA with a nucleotide composition similar to that of DNA had been demonstrated, proof was still lacking that these RNAs were perfect copies of DNA – informational RNA in Spiegelman's words. Together with Ben Hall, he demonstrated this in 1961 (Hall and Spiegelman 1961). Spiegelman immediately recognized the numerous potential applications of the new technology to characterization of the relations between the RNAs present in cells and organisms and DNA. With Ferruccio Ritossa, he showed that the DNA complementary to the ribosomal RNA was present in the nucleolus organizer region of *Drosophila* (Ritossa and Spiegelman 1965).

The most promising application of the new technology was in the study of differentiation and development. A comparison was made of the RNA molecules present in the different tissues of an adult organism (McCarthy and Hoyer 1964). The question of the relative roles of the nucleus and

Keywords. HnRNA; provirus; splicing

Published online: 27 January 2014

of the cytoplasm in development had been central to embryology since the beginning of the twentieth century. In the new molecular framework, the issue has been transformed into an estimation of the relative roles of maternal RNA present in the oocyte and newly synthesized RNA in the early stages of development. Inhibitors of transcription and protein synthesis had been used to answer this question, but the results were ambiguous, partly due to the impossibility of applying the inhibitors for long periods of time. Herman Denis was one of the first to address this question for amphibian development (Denis 1966). Refinements of the hybridization technique were necessary: competition between different populations of RNA, and saturation experiments to estimate the amount of DNA transcribed into RNA. Similar studies were done by Eric Davidson's group (Davidson *et al.* 1968). This work revealed the existence of highly different concentrations of RNA molecules and progressively led to the classification of RNAs according to their abundance.

A similar result emerged from the study of DNA-DNA hybridization. Some repeated sequences renatured very rapidly, whereas unique DNA sequences required days to anneal efficiently. In this case also, a distinction was introduced between different categories of more or less repeated sequences. The conclusions of these early studies were firmly established by Roy Britten and David Kohne in 1968 (Britten and Kohne 1968).

It was possible to 'hybridize' the DNA-DNA and DNA-RNA hybridization experiments: did the abundant RNA sequences correspond to repeated DNA, or to unique DNA sequences?

In parallel, DNA-DNA annealing was used to estimate the genetic distance between different species, which could be related to a decrease in the temperature of denaturation of the hybrid molecules formed with DNA of the two species, compared with the homologous DNA molecules. Early experiments showed a difference greater than expected from the comparison of amino acid sequences, but which was explainable if most of the mutations occurred at the third position of the codons (having a limited impact on amino acid sequence because of the degeneracy of the code), or in non-coding DNA sequences.

Particular attention was paid to the genetic relations between humans and primates (Kohne *et al.* 1972; Hoyer *et al.* 1972). Chimpanzees were shown to be our closest relatives. The small genetic distance between humans and chimpanzees was not emphasized in these articles, and had to wait for King and Wilson's famous publication of 1975 reporting the use of multiple approaches, including DNA-DNA hybridization (King and Wilson 1975).

Work continued through the 1970s (Galau *et al.* 1974, 1976, 1977) with increasingly sophisticated techniques. For instance, rapidly annealing repeated DNA sequences could be eliminated by adsorption on hydroxyapatite; cDNA could be produced from RNA, facilitating the competition experiments; RNA could be isolated from polysomes. These technologies were used to explore new organisms for which there was a total lack of information on the organization of the genome and on the molecular processes of differentiation and development (see, in the case of *Trypanosoma cruzi,* Lanar *et al.* 1981). Cellular systems able to differentiate *ex vivo*, such as some cell lines derived from teratocarcinoma, or myoblasts prone to differentiate into myofibrils, were actively studied (see, for instance, Affara *et al.* 1977).

3. Difficulties encountered in the hybridization experiments

Interestingly, the possibility of DNA-DNA or DNA-RNA hybridization with previously denatured eukaryotic DNA was a surprise: most researchers expected that the complementary sequences would not be able to meet and anneal in such a complex mixture of different sequences. The fact that it worked does not mean that it did so reliably: the published results often differed.

The origins of this difficulty in obtaining reproducible results with this technology were precisely described very early (McCarthy and Church 1970). The conditions of annealing – pH, temperature, ionic strength – were crucial for the success of the experiments, and often differed from one experiment to another. Even more problematic was the fact that the conditions optimal for one sequence (percentage of GC and number of copies) were not optimal for another sequence (different GC content, different abundance). In addition, the experiments were long – days for unique sequences – and performed at high temperatures conducive to degradation of the biological material. Finally, the distinction between different categories of DNA or RNA sequences (highly repeated or not, very abundant or not) remained partly arbitrary, the cut point being chosen by the experimenter. To summarize in a few words, much more was expected from these technologies than they were able to provide!

More than the difficulties encountered in the reproduction of the results, and the obvious limits of these techniques, two major failures explain their abandonment at the beginning of the 1980s. The hybridization experiments of Howard Temin with Rous sarcoma virus (Temin 1964) did not provide convincing evidence of the existence of a DNA provirus, and ten more years were necessary before his hypothesis was accepted. The numerous comparisons between heterogeneous nuclear RNA and cytoplasmic (messenger) RNA by molecular hybridization did not lead to the discovery that the initial transcription products were spliced in the nucleus to generate the mature messenger RNA.

Concerning Rous sarcoma virus, the obstacles were not the alleged dominance of the central dogma of molecular biology, or the experiments of Spiegelman (Doi and Spiegelman 1962) showing that there were no sequences in the bacterial chromosome that were complementary to the RNA virus MSΦ2, and therefore his demonstration that an RNA virus could replicate without a DNA template. The only obstacle was the poor quality of the hybridization experiments performed by Temin, their low signal-to-noise ratio (Marcum 2002; Fisher 2010).

As soon as nuclear RNAs were discovered, their relation with cytoplasmic RNAs was explored (Birnboim *et al.* 1967), but conflicting observations accumulated in the following years (Wold *et al.* 1978). Some precise models of a structural relation between HnRNA and mRNA emerged (such as in Weinberg 1973), but no one anticipated the discovery of 1977. The latter was made possible by the new tools of genetic engineering: distant (in the genome) DNA fragments obtained by the action of restriction enzymes were shown to hybridize to the same mRNA molecule (see Witkowski 1988, for a precise historical description of the discovery of split genes). In addition, hybridization experiments wrongly suggested that the same HnRNAs were shared by different cell types, and that the main level of control during development and differentiation was not the production of nuclear RNA, but that of cytoplasmic RNA (Kleene and Humphreys 1977; Chikaraishi *et al.* 1978).

The complexity of this type of hybridization experiments, and the conflicting results that they generated, explain why many researchers and labs were reluctant to develop it and preferred to adopt techniques that were able to provide a precise identification of the molecular species under study: *in vitro* translation of mRNAs, and identification of proteins by 2D gel electrophoresis and specific antibodies; in *ex vivo* differentiation experiments, characterization of proteins or of the activities of newly synthesized enzymes.

At the beginning of the 1980s, the tedious characterization of populations of RNA was replaced by the cloning and characterization of developmental genes – homologues of the genes that had been described in *Drosophila* through careful genetic studies of development. *In situ* hybridization experiments rapidly developed: in contrast to the molecular hybridization experiments described before, they targeted one specific DNA or RNA sequence, and required for their completion the prior cloning of these sequences.

The scepticism expressed by some developmental biologists in the early 1980s regarding the positive impact on embryology of molecular studies rapidly vanished: one of the roots of this scepticism was probably the abundance of complex and conflicting results obtained by molecular hybridization.

The fascination for repeated sequences also became unfashionable when it was proposed in 1980 that these sequences were parasitic, selfish DNA, not eliminated by natural selection (Doolittle and Sapienza 1980; Orgel and Crick 1980).

4. Discussion and conclusion

The transient interest in molecular hybridization experiments stemmed from the lack of appropriate molecular techniques for the study of eukaryotes and their development in the 1960s and at the beginning of the 1970s. Molecular hybridization was the only molecular technique available during this period. Interestingly, the numerous observations that had been made on the abundance of nuclear transcripts were forgotten, and only recently rediscovered and confirmed.

The progressive renunciation of molecular hybridization experiments in favour of the study of specific developmental genes can also be considered as a transition from an analogue description of development to a digital one, at the same time that analogue machines were being replaced by digital computers. What became important were the precise bits of information, not a global estimate of this information.

Molecular hybridization experiments induced in researchers, two different and opposed attitudes. Some were fascinated by their sophistication as well as by the quantification of the results. Others were repelled by this complexity and the apparent inconsistency of the results, by the arbitrariness in the design of the experiments and in the interpretation of the results. Some of the leading groups working on differentiation and development never used them, or only parsimoniously, often in collaboration with other labs – just to see! Such was the case of Sydney Brenner, François Jacob and David Yaffe.

I would like to suggest that such contrasted attitudes among biologists recurrently emerge as soon as a new, quantitative approach to biological phenomena emerges. Some are fascinated by the novelty, richness and complexity of the observations that can be generated. They expect the emergence from the data of a new 'logic of life'. Others prefer to focus on well-established facts, and consider with suspicion generalizations built on still preliminary and conflicting data.

Acknowledgements

I am indebted to David Marsh for his critical reading of the manuscript, and to the anonymous reviewers for their helpful comments.

References

Affara NA, Jacquet M, Jakob H, Jacob F and Gros F 1977 Comparison of polysomal polyadenylated RNA from embryonal carcinoma and committed myogenic and erythropoietic cell lines. *Cell* **12** 509–520

Birnboim HC, Pene JJ and Darnell JE 1967 Studies on HeLa cell nuclear DNA-like RNA by RNA-DNA hybridization. *Proc. Natl. Acad. Sci. USA* **58** 320–327

Britten RJ and Kohne DE 1968 Repeated sequences in DNA. *Science* **161** 529–540

Chikaraishi DM, Deeb SS and Sueoka N 1978 Sequence complexity of nuclear RNAs in adult Rat tissues. *Cell* **13** 111–120

Davidson EH, Crippa M and Mirsky AE 1968 Evidence for the appearance of novel gene products during amphibian blastulation. *Proc. Natl. Acad. Sci. USA* **60** 152–159

Denis H 1966 Gene expression in amphibian development II. Release of the genetic information in growing embryos. *J. Mol. Biol.* **22** 285–304

Doi RH and Spiegelman S 1962 Homology test between the nucleic acid of an RNA virus and the DNA in the host cell. *Science* **138** 1270–1272

Doolittle WF and Sapienza C 1980 Selfish genes, the phenotype paradigm and genome evolution. *Nature* **284** 601–603

Doty P, Marmur J, Eigner J and Schildkraut C 1960 Strand separation and specific recombination in Deoxyribonucleic acids: physical chemical studies. *Proc. Natl. Acad. Sci. USA* **46** 461–476

Fisher S 2010 Not beyond reasonable doubt: Howard Temin's Provirus hypothesis revisited. *J. Hist. Biol.* **43** 661–696

Galau GA, Britten RJ and Davidson EH 1974 A measurement of the sequence complexity of polysomal messenger RNA in sea urchin embryos. *Cell* **2** 9–20

Galau GA, Klein WH, Davis MM, Wold BJ, Britten RJ and Davidson EH 1976 Structural gene sets active in embryos and adult tissues of the sea urchin. *Cell* **7** 487–505

Galau GA, Lipson ED, Britten RJ and Davidson EH 1977 Synthesis and turnover of polysomal mRNAs in sea urchin embryos. *Cell* **10** 415–432

Giacomoni D 1993 The origin of DNA:RNA hybridization. *J. Hist. Biol.* **26** 89–107

Hall BD and Spiegelman S 1961 Sequence complementarity of T2 DNA and T2 specific RNA. *Proc. Natl. Acad. Sci. USA* **47** 137–146

Hoyer BH, van de Velde NW, Goodman M and Roberts RB 1972 Examination of Hominid evolution by DNA sequence homology. *J. Hum. Evol.* **1** 645–649

King MC and Wilson AC 1975 Evolution at two levels in Humans and Chimpanzees. *Science* **188** 107–116

Kleene KC and Humphreys T 1977 Similarity of hnRNA sequences in blastula and pluteus stage sea urchin embryos. *Cell* **12** 143–155

Kohne DE, Chiscon JA and Hoyer BH 1972 Evolution of primate DNA sequences. *J. Hum. Evol.* **1** 627–644

Lanar DE, Levy LS and Manning JE 1981 Complexity and content of the DNA and RNA in *Trypanosoma cruzi. Mol. Biochem. Parasitol.* **3** 327–341

Marcum JA 2002 From heresy to dogma in accounts of opposition to Howard Temin's DNA Provirus hypothesis. *Hist. Phil. Life Sci.* **24** 165–192

Marmur J and Lane D 1960 Strand separation and specific recombination in Deoxyribonucleic acids: biological studies. *Proc. Natl. Acad. Sci. USA* **46** 453–461

McCarthy BJ and Hoyer BH 1964 Identity of DNA and diversity of messenger RNA molecules in normal mouse tissues. *Proc. Natl. Acad. Sci. USA* **52** 915–922

McCarthy BJ and Church RB 1970 The specificity of molecular hybridization reactions. *Annu. Rev. Biochem.* **39** 131–150

Orgel LE and Crick FHC 1980 Selfish DNA: the ultimate parasite. *Nature* **284** 604–607

Rich A 1960 A hybrid helix containing both deoxyribose and ribose polynucleotides and its relation to the transfer of information between the nucleic acids. *Proc. Natl. Acad. Sci. USA* **46** 1044–1053

Ritossa F and Spiegelman S 1965 Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* **53** 737–745

Spiegelman S and Kamen MD 1946 Genes and nucleoproteins in the synthesis of enzymes. *Science* **104** 581–584

Temin HM 1964 Homology between RNA from Rous Sarcoma Virus and DNA from Rous Sarcoma Virus-infected cells. *Proc. Natl. Acad. Sci. USA* **52** 323–329

Weinberg RA 1973 Nuclear RNA metabolism. *Annu. Rev. Biochem.* **42** 329–354

Witkowski JA 1988 The discovery of 'split' genes: a scientific revolution. *TIBS* **13** 110–113

Wold BJ, Klein WH, Hough-Evans BR, Britten RJ and Davidson EH 1978 Sea urchin embryo mRNA sequences expressed in the nuclear RNA of adult tissues. *Cell* **14** 941–950