A Survey of Dynamical Genetics

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Abstract. The classical view of genetics is based on the central dogma of molecular biology that assigns to DNA a fundamental but static role. According to the dogma, DNA can be duplicated only in identical copies (except for random errors), and no smart mechanism can alter the information content of DNA: in more detail, the direction of transfer of the genetic information is only from DNA through RNA to proteins and never backwards.

However, starting from the so-called dynamic genome (McClintock's jumping genes), and the so-called dynamic mutations (such as the trinucleotide expansion or, more generally the instability of the number of tandem repeats of longer sequences), there is now a growing body of important cases where it is known that the DNA is altered in a more or less sophisticated way, often by smart enzymatic mechanisms.

The study of all such dynamic phenomena and of their interpretations can be naturally called dynamical genetics.

In this survey we examine a number of such dynamic phenomena, and also some phenomena of great biological importance that have no universally accepted explanation within a static approach to genetics, and for which a dynamical interpretation has been only proposed. Important examples are some controversial but interesting phenomena such as horizontal transmission and Creutzfeldt-Jacob Disease, and those peculiar DNA structures known as G-quadruplexes.

1. INTRODUCTION

In this paper we consider a number of phenomena of great biological importance that cannot be explained by the "central dogma of molecular biology", which can be worded as follows: DNA can be duplicated only in identical copies (except for random errors), the direction of transfer of the genetic information is only from DNA through RNA to proteins and never backwards, and no purposive mechanism can alter the information content of the DNA.

In a genome thought of as purely static the genes are stored in a chromosome along the double helix DNA (1953), they are inherited according to simple mendelian-like laws (1865), and changed only by blind random mutations that successively, generation by generation, undergo a darwinian selection.

However in the second half of 20th century (Thieffry and Sarkar [1998]) a number of phenomena has been discovered having surprising features not explainable by means of the known laws of genetics, but that exhibit behaviours that can well deserve to be called dynamic.

Starting from early fifties Barbara McClintock has thoroughly studied the maize genetics discovering some mobile elements, i.e. DNA sequences that can move inside a chromosome from one site to another, or from one chromosome to another: the so-called transposable elements or jumping genes. McClintock stated that the genome of an organism is dynamic – and not static – and that its becoming is stimulated by the environment, thus introducing the model of the so-called "dynamic genome" (Fedoroff and Botstein [1992]). While initially the jumping genes were studied mainly for plants, they are now considered important for all living beings (Lönnig and Saedler [2002], Johanning *et al*. [2003]).

In the seventies Susumu Tonegawa discovered that the antibody diversity in the immune system is due to a genetic diversity generated by somatic recombination. We shall discuss below this phenomenon called V(D)J recombination and similar dynamic phenomena such as those discovered by Max D. Cooper in the eighties for the immunoglobulin class-switching.

In the early nineties peculiar DNA instabilities of tandem repeated sequences

(trinucleotide expansion) have been detected that revealed (see below) to be the aetiology of some genetic diseases, such as Fragile X Syndrome, Myotonic Dystrophy, Huntington Chorea. Richards and Sutherland [1992] first introduced the term of dynamic mutation to describe mutations that can cause (or predispose to) other mutations.

In the late nineties it has been discovered for some of these diseases that instability occurrs not only from parent to offspring but also within the same organism, with a possibly different degree of expansion for different cells.

Almost all the above phenomena concern humans or at least superior organisms; but highly dynamic mechanisms that act on genome have a fundamental role in parasites (viruses, bacteria, and protozoa). In particular some viruses interact dynamically with the human genome using integration or retrotranscription. Also some other parasites alter their genome to mutate the surface antigenic determinants in order to evade the host immune system.

It is interesting to note that, while often the genome is static and DNA assumes the classical double-helix structure, surprisingly, in cases where the genome is dynamic, very often peculiar DNA structures are involved, that link four DNA strands, the so-called quadruplexes, as detailed below.

Although many of the above cases are related to some pathologies, there is now a growing body of important physiological cases where it is known that the DNA sequence is altered in a more or less sophisticated way, often by smart enzymatic mechanisms; in more detail there exist smart purposive mechanisms that monitor and regulate the length of some tandem repeated sequences or that for example cause rearrangements, point mutations and apoptosis; for brevity's sake the collective acronym TRLM (Tandem Repeat Length Manager) has been coined for all such mechanisms (De Fonzo *et al*. [2000], De Fonzo *et al*. [2001]). A recently emerging viewpoint is that pathological cases are simply due to a malfunction of normally physiologic mechanisms.

As for the biochemical basis of such mechanisms – particularly studied in man and in *S. cerevisiae* (D'Amours and Jackson [2002]) – we note that they are usually implemented by enzymatic complexes of many different proteins, while in simple metabolic reactions enzymes are usually made of one or very few subunits; moreover in the first case (TRLM) each protein is used in many different complexes for many different reactions, while in the second case (metabolic reactions) usually there is a one-to-one correspondence between reactions and enzymes, and every protein is used in a single complex.

For example, in conjunction with many different sets of proteins, a complex of three proteins, the Mre11/Rad50/Xrs2, performs a surprisingly large range of functions in yeast (Haber [1998]), and is also involved in CAG trinucleotide repeat expansions in a yeast model (Richard *et al*. [2000]), an expansion which is neither mitotic nor meiotic. A similar phenomenon – already hypothesised in (De Fonzo *et al*. [2000]) – has now been generally shown for trinucleotides instability in human neurodegenerative diseases (Richards [2001]). Another very important example is the case of the Ku proteins that are involved in a large number of different basic events (Tuteja and Tuteja [2000], Schild-Poulter *et al*. [2003]).

It has been also hypothesised (De Fonzo *et al*. [2000]) that some TRLM actions are performed by enzymes composed of proteins associated with oligonucleotides (as in telomerase). Now many researches concern the so-called microRNAs, short tracts of RNA that cooperate with suitable proteins editing other RNA molecules and modulating gene expression (Nelson et al. [2003]), while their involvement in some TRLM remains till now, as far as we know, hypothetical.

The recently proposed term *dynamical genetics* (De Fonzo *et al*. [2000], De Fonzo *et al*. [2001]) is used here in order to provide a common framework for all the above dynamic phenomena, their interpretation, and the mechanisms generating them.

We note that, while till now we have considered mechanisms that manage the genome on an individual time scale, if we switch to geological time scales the recombination mechanism is also a driver force for the genome evolution (Posada *et al*. [2002]). In particular a very special kind of smart mechanism, involved in protein evolution, is the construction of new genes by assembly of preexisting tracts of genes (each one coding for a functional module) (Ejima and Yang [2003]), in a bricolage-like way, in the formation of organisms of increasing complexity (Lavorgna et al. [2001]). It is interesting to point out that a bricolage-like behaviour occurs also in the immune system, as described in more detail below.

We finally mention here (and shall detail below) a theory, explicitly inspired to dynamical genetics, that has been recently proposed to explain the CJD. According to this theory (unlike the best-known protein-only theory) the contagion would be due to specific oligonucleotides that are carried by prions.

2. DYNAMICAL PHYSIOLOGIC MECHANISMS

Among the many possible examples, we consider here only a few well studied mechanisms of human relevance.

We first examine the best known human physiologic mechanisms, whose existence is undisputed and that can be unquestionably called dynamical.

- **The chromosomal crossing over**. During meiosis (or more exactly during pachytene in prophase of meiosis I) homologous chromosomes may recombine by interchange of homologous tracts. This is due to chromosome pairing in a number of sites and is of fundamental importance; it is the best known case of DNA rearrangement and may well be interpreted as a first described example (Morgan and Cattel, 1912) of a dynamical genetics phenomenon.

- **The regulation of telomeres length** (Rhodes et al. [2002]). When a duplication occurs in a chromosome, the terminal DNA tracts, the telomeres, become shorter since they loose the last nucleotides; although the telomeres are non-coding, such a process, if not countered, is unavoidably harmful when endlessly iterated. In many cells the telomerase action or other mechanisms regulate the telomeres length by increasing it again.

- **Phenomena involving immunoglobulin genes.** Immunoglobulin genes are involved in some characteristic dynamic phenomena controlled by mechanisms that are still not completely known.

  - **The immunoglobulin heavy chain class switching recombination.** In an immunoglobulin molecule (consisting of a number of heavy and light polypeptide chains), a part of the heavy chain (together with a part of the light chain) is specific against a particular antigen, while another part of the heavy chain determines alone the class, which corresponds to different features such as the destination: for example the immunoglobulin molecules in class A (IGA) are mucosa-specific, while those in class G (IGG) are plasma-specific.

    The capability of the immune system to induce some lymphocytes to switch from one class to another (while preserving the specificity against the same antigen) enhances the efficiency of the performance in fighting against parasites. However, since the two above parts of the heavy chain are coded by the same gene, this capability is implemented by means of a true editing mechanism, acting (not directly on the protein

but) on the coding gene, thus changing only the class (and therefore e.g. the destination as IGA and IGG) and not the antigen specificity (Manis *et al*. [2002]).

- **The V(D)J recombination** (Lewis [1994]). The immune system is capable of producing a huge number of different immunoglobulins and of T-cell receptors but each mature lymphocyte can produce only one of them. The genes that code for such molecules are present only in mature lymphocytes and can be different in different lymphocytes.
  To produce such genetic diversity the basic mechanism is a special somatic site-specific DNA rearrangement, occurring during lymphocyte maturation, as follows.
  In immunoglobulins, the rearrangement involves tracts belonging to three different types, named, for historical reasons, V (Variable), D (Diversity) and J (Joining), and each one of such tracts is present in a number of similar contiguous copies. To assemble a DNA tract to synthesise an immunoglobulin chain, two or three tracts of the above types are randomly selected and joined. For the light chain only a V tract and a J tract are joined, while for a heavy chain V, D, and J tracts are joined. Such editing-like mechanism is called V(D)J recombination.
  The recombination of genes coding for T-cell receptors occurs in a similar way.

- **The homogenisation and the hypermutation in immunoglobulin genes.** The immunoglobulin genes contain constant and variable tracts. The constancy of the constant tracts is suitable to preserve some functions; while each variable tract contains a number of hypervariable tracts that enhance the genetic diversity between lymphocytes (Kelsoe [1999]). Both phenomena are obviously of essential relevance, and, although for them no satisfactory explanation is as yet available, a dynamic interpretation has often been proposed, based on a specific gene conversion: in more detail the constancy is ascribed to homogenisation phenomena between equal tracts (Bailey et al. [1998]), and the variability is ascribed to the mixing up of different tracts (Harris *et al*. [1999]).

• **The management of the copies of rDNA transcriptional units**. The transcription of nearly all genes produces a number of mRNA molecules and each of them produces a number of polypeptides, so that a great rate of polypeptide synthesis is allowed. The ribosomal RNA is one of the rare exceptions, since it is produced by transcription of rDNA but does not undergo translation; this lack could cause too low a yield that is balanced upstream by a great number of copies of rDNA genes in the genome, thus coping with the high rate of production of rRNA often required by the metabolic needs.

- A first dynamic feature is the fact that such a number of copies is variable, regulated by some basically unknown smart mechanism, according to the current needs (Gonzalez and Sylvester [1995]).
- A second feature, usually interpreted as dynamic, is the fact that such copies remain exactly identical, although one could expect that they would tend to diverge functionally due to random point mutations (Liao [1999]).

Beside these surely existing mechanisms, other mechanisms of dynamical genetics nature have been hypothesized in humans. A very interesting example is given by the hypothesized purposive mechanisms, based on the management of the DNA, put forward to explain the basis of long-term memory: mechanisms similar to V(D)J recombination (Peña De Ortiz and

Arshavsky [2001]), meiotic mechanisms of recombination (Dietrich and Been [2001]), mechanisms that drive specific VNTRs or genomic rearrangements (De Fonzo *et al*. [2000]).

Other interesting examples of dynamical genetics mechanisms act in some parasites.

Some viruses interact dynamically with the human genome making use of mechanisms such as integration, as Epstein-Barr Virus (Gualandi *et al*. [1995]), or retrotranscription followed by integration, as HIV (see below).

Some other parasites, eukaryotes as *Plasmodium*, and bacteria as *Mycobacterium* and *Mycoplasma*, dynamically alter their genome, for example via some genomic rearrangements or VNTRs (Yeramian and Buc [1999]), to mutate the surface antigenic determinants in order to evade the host immune system (Lysnyansky *et al*. [2001], van Belkum *et al*. [1998], Moxon *et al*. [1994], Borst [1991]).

3. THE LOCATION OF GENES IN THE GENOME

In the classical genetics (since the researches of Morgan), i.e. in a genome considered devoid of dynamic mechanisms except the crossing over, the location of the genes along a chromosome was considered immaterial except just in the crossing over case. However, in the last years some cases have been discovered where the gene order is not random and even is sometimes crucial, so indirectly confirming the need of dynamic interpretations.

The first example of non random order is provided by fact that the homeobox genes (which are regulatory genes that control the development of various animals) are ordered along a chromosome, according to their expression pattern along the organism (Gaunt and Singh [1990]) and to their chronological activation (Watson [2001]).

An example of the importance of the gene order is provided by an experiment that has shown that altering the order of globin genes impairs a correct gene expression during development (Hanscombe *et al*. [1991]).

More recently, the study of the gene position has acquired applicative implications in the road from genomics to proteomics (Han *et al*. [2002]). Since we know a huge number of gene sequences, but we ignore the features of a great number of coded proteins, the greatest problem is to predict, for the sequenced genes, the functions of the coded proteins and the related networks (Gavin and Superti-Furga [2003]).

In order to guess functions and interactions of a protein encoded by a given gene, the standard assumption is to attribute similar functions to similar genes; a new promising idea is to assume that the neighbouring genes probably code for proteins working in tandem (or at least in the same network) (Overbeek *et al*. [1999]). When different proteins are coded in the same mRNA (i.e. when dealing with a polycistronic operon) this functional relation usually holds, but polycistronic operons are common only in bacteria and in nematodes (von Mering and Bork [2002]). While in the case of simply close genes, clustered in the so-called über-operons (Lathe *et al*. [2000]), a functional relation seems to hold (but this is till now controversial) albeit in a more complicated way (Huynen *et al*. [2001]): Lercher *et al*. [2002] maintain that this phenomenon could be spurious.

4. VNTRs

Main actors of the dynamical genetics are the so-called VNTRs, a particular case of Tandem Repeat (TR). TRs are tracts, abundant in the genomes of many species, where a unit is tandemly repeated, exactly or nearly exactly, a number of times. The VNTRs (variable

number of tandem repeats) are TRs where the number of repetitions is variable: possibly within a population (polymorphism), or from parent to offspring (genetic instability), or within the same organism (somatic instability causing mosaicism).

A TR with a short repeated unit (say 2-4 bases) is usually also called microsatellite, and, with a longer unit, minisatellite (Chambers and MacAvoy [2000]). The most studied TRs are trinucleotides, due to their frequent involvement in human pathologies (Astolfi *et al.* [2003]).

We consider here in more detail VNTRs in humans, since, apart from their obvious interest, some diseases and some important features occur almost only in humans (Bois [2003]). Initially, VNTRs were considered merely variable within a population, almost always with neither physiological nor pathological relevance, and were only used as markers for genetic studies or forensic applications (Nakamura *et al*. [1998]).

Successively (Richards and Sutherland [1992]), some VNTRs have been identified as responsible of a number of human diseases, showing peculiar genetic instabilities and other peculiar features. These diseases (as, for example, Huntington Disease and some chromosomal fragilities) are often both neurodegenerative and caused by an excessive number of tandem repeats of trinucleotides (Jasinska et al. [2003]) or sometimes of units longer than three (Virtaneva *et al*. [1997], De Fonzo *et al*. [1998], Wells *et al.* [1998]).

Moreover for many of these diseases such a number often undergoes an expansion from parent to offspring (the so-called anticipation), and few repetitions correspond to absence of symptoms, while more repetitions correspond to more severe symptoms or earlier onset (La Spada *et al*. [1994], Sutherland and Richards [1995], Warren and Ashley [1995]); other peculiar common features are neuronal loss by apoptosis without detected metabolic abnormalities (La Spada *et al*. [1994]), and late onset.

Some VNTRs have been found to be an important predisposing cause for other disorders, for example cancer (Ionov *et al*. [1993], Aquilina and Bignami [2001], Oda *et al*. [2002]), diabetes (Bell [1981], Vafiadis *et al*. [1997]), mental disorders (Ueno [2003]).

The TR expansion is often considered as a harmful side effect of other mechanisms that do not have as their primary function to manipulate VNTRs: examples are DNA replication (Kunkel [1993]), DNA repair (de la Chapelle and Peltomäki [1995]), unequal cross-over (Jeffreys *et al*. [1998]) and transposon homing (Pardue *et al*. [1996]).

A somatic variability of TRs has been detected in the last years, especially for a number of pathogen VNTRs; moreover a growing number of evidences of physiological role of a somatic variability of some TRs are being discovered (Li *et al*. [2002] and references therein).

We finally note that human VNTRs known to be useful are still few, while in some parasites VNTRs known as useful are more common (see above).

5. DNA QUADRUPLEXES

Among the factors that are involved in the dynamical behaviour of DNA, of growing importance is becoming the presence of the so-called quadruplexes.

Quadruplexes are peculiar (non double-helix) DNA structures, that link four DNA strands stabilised by some square planar arrangements (quartets), each one made of four bases. The most frequent and interesting (and also the thermodynamically most stable) cases are when the four bases are guanines, and in this case the planar arrangements are called G-quartets (Gellert et al. [1962], Keniry [2000], Shafer and Smirnov [2000], Simonsson [2001]).

The existence also of quadruplexes with quartets containing non-guanine bases has been now demonstrated in a number of cases (Howell and Usdin [1997], Patel *et al.* [1999],

Patel *et al*. [2000]), some of which of medical relevance (Usdin and Grabczyk [2000]), and in other cases only hypothesized (De Fonzo *et al*. [2001]); recently structures formed by a G:C:A:T tetrad have been found in solution (Escaja *et al*. [2003]).

G-quartets exhibit unique chemical-physical characteristics also outside DNA: for example, in crystal form, they cause the reflectivity of fish scales (Rowe and Denton [1997]) or the shining of the retina of nocturnal animals; and they are also used in technology, such as in nacreous pigment manufacturing and in biomolecular nanotechnologies (Calzolari *et al*. [2002], Alberti and Mergny [2003]).

An example of a non-conventional behaviour of DNA is provided by aptamers. Aptamers are oligonucleotides (DNA or RNA) that specifically bind to some enzymatic sites or to other proteins. Since almost always aptamers contain DNA or RNA quadruplexes, it is conceivable that quartets give an aptamer the rigidity required for molecular docking specificity.

As for the dynamical behaviour of DNA in biology, there exist in fact many cases where it is already accepted that quadruplexes play a fundamental role in physiological or pathological genome dynamics (Arthanari and Bolton [2001]): as a typical example we quote the fact that quadruplexes often induce genetic recombinations.

We note that the main features of a DNA quadruplex have been till now thoroughly studied *in vitro*, while the very existence of quadruplexes *in vivo* is often only indirectly argued, although with a growing confidence. Siddiqui-Jain *et al*. [2002] claim the detection of a physiological role of a DNA quadruplex in human cells.

Among the examples of physiological genome dynamics quoted in section 2, quadruplexes have certainly a role at least in the following three cases:

- **Management of telomere length**: telomeres contain VNTRs that form quadruplexes (Williamson [1994]), and telomere length regulation is due to the inhibition of telomerase by suitable quadruplexes (Zahler *et al*. [1991]).
- **Class switching of the immunoglobulin heavy chain**: each genome region where class switching occurs (switch region) contains a VNTR that is different for each class type and forms a quadruplex structure (Sen and Gilbert [1988]).
- **Chromosomal crossing over:** The pairing of homologous chromosomes and the subsequent crossing over is caused by quadruplexes (Sen and Gilbert [1988]) enzymatically formed and afterwards enzymatically broken (Liu and Gilbert [1994]).

There exist also cases where quadruplexes are the cause of important diseases (possibly as a result of a malfunctioning in some physiological role). Well-known examples are the following.

The causal role of quadruplexes is already generally accepted in some of the VNTR-associated diseases quoted above, as Progressive Myoclonus Epilepsy Type-1 (Saha and Usdin [2001]), Fragile X Syndrome (Fry and Loeb [1994]), and Human Type I Diabetes (Simonsson [2001]). For many such diseases quadruplexes form just inside VNTRs (of DNA, or sometimes of RNA). In the case of Fragile X Syndrome the link between the quadruplexes and the disease is more complex: while the chromosomal fragility seems to be due to the formation of quadruplex structures in DNA, the mental retardation seems to be due to the fact that Fragile X Mental Retardation Protein (FMRP) physiologically binds to intramolecular quadruplexes of mRNA of unknown proteins (Darnell *et al*. [2001]).

Other quadruplex-related diseases are Bloom and the Werner syndromes: the cause of these diseases is the lack of the respective (Bloom's or Werner's) helicase apt to unfold specific quadruplex structures (Sun *et al*. [1998], Fry and Loeb [1999]).

Quadruplexes are also probably involved in the harmful action of some parasites, although their role in genome dynamics is still partially unclear; we quote three important examples of human relevance:

- The dimerisation of the RNA of the Human Immunodeficiency Virus type 1 (Awang and Sen [1993]). We note, by the way, that the integration of HIV-1 is inhibited from suitable oligonucleotide drugs that form G-quartets (Mazumder *et al*. [1996]); and it has been proposed that integrase interacts with the quadruplexes of the virus and therefore the drug efficacy is an example of competitive enzymatic inhibition (De Fonzo *et al*. [2001]).
- The integration of Epstein-Barr virus, even if the involvement of quadruplexes is still convincingly suggested only by some indirect evidences (Sun *et al*. [1997], De Fonzo *et al*. [2001]).
- The antigenic variation in *Trypanosoma brucei* (Eid and Sollner-Webb [1997]).

Another dynamical mechanism where quadruplexes appear to be involved is their folding and unfolding in transcription factor promoters, a phenomenon that until recently was completely ignored, but that has been now recognised as having an important regulating function for cell metabolism and physiology, via the modulation of the transcription factor synthesis. Simonsson was the first to state (providing some experimental evidence) that inside the promoter of human c-*myc* (an important transcription factor), a structure transition may occur from double helix to quadruplex (and vice versa), and that this switching modulates the transcription rate (Simonsson *et al*. [1998]). Hurley and coworkers, for the first time in human cells (according to Borman [2002]) provided further and stronger experimental evidence, based on in vivo experiments (Siddiqui-Jain *et al*. [2002]).

In more general terms Simonsson *et al*. [1998] point out that G-rich sequences are also present inside the promoter of nearly all the main transcription factors (oncogenes), and maintain that also in these cases the quadruplexes may have an analogous regulation role. We note that, since transcription factors regulate the transcription of most genes, the above hypothesis amounts to state that quadruplexes may have, directly or indirectly, a very general regulation role.

We note that such role may be even more general: in fact G-rich sequences are present not only inside the promoter of transcription factors, but also inside the promoter of some genes involved in dynamic phenomena of DNA, for example in the case of autoantigen Ku (Ludwig *et al*. [1997]). It has been therefore proposed (De Fonzo *et al*. [2001]) that quadruplexes may have a regulation action not only on the promoters of the main transcription factors but also on the promoters of a number of genes of crucial importance for dynamical genetics; similar considerations are reported by Borman [2002].

We add as a side remark that many transcription factors also contain VNTRs. For a discussion on the probable dynamical importance of VNTRs in transcription factors and its possible implications in ageing, see De Fonzo *et al*. [2000].

Practical applications of the above findings in the pharmacological field have already been considered:

- since management of telomere length is due to the inhibition of telomerase by suitable quadruplexes, drugs interacting with the quadruplexes in telomeres have been proposed to counter ageing (allegedly caused by the telomeres shortening) and cancer (allegedly allowed by the telomeres length control) (Mergny and Helene [1998], Riou *et al*. [2002]);
- more recently, since quadruplexes appear to regulate oncogenes, drugs interacting with the quadruplexes in the promotors of the oncogenes (see Borman [2002] and references quoted therein) are being investigated as antiproliferatives;
- it has been shown that using as drugs just some oligonucleotides prone to form quadruplexes, one obtains an antiproliferative effect, particularly promising against

restenosis (Burgess *et al*. [1995]) and proliferation of tumoral cells (Dapic *et al*. [2003]); and since further experiments have shown that such quadruplexes end up in the nucleolus, it has been suggested (Bates *et al*. [1999]) that this very fact slows down the proliferation. Simonsson and Henriksson [2002] have put forward the following less demanding hypothesis: if the oncogene transcription rate is driven by the interaction between specific molecules and the quadruplexes in the promoter, drugs containing similar quadruplex structures can divert many of those molecules from interacting with the promoter, obtaining consequently an antiproliferative effect;

- it has been found that some specific oligonucleotides, that are powerful inhibitors of HIV-1 integrase, form quadruplex structures (Jing *et al*. [2000]).

For the sake of completeness we note that a number of (usually pathological) recombinations is sometimes associated to the presence of triple helix structures. For a discussion see De Fonzo *et al*. [2001]. We note that there exist also structures involving up to 7 strands (Suhnel [2001]). All such unusual structures do not seem, as far as we know, to have any significant dynamical role comparable to quadruplexes.

6. GENOMIC IMPRINTING, ADAPTIVE MUTATIONS, HORIZONTAL TRANSMISSION AND DNA UPTAKE

The term "genomic imprinting" is used when, depending on maternal or paternal origin, a specific non-mendelian difference occurs in the behaviour of one or more genes. The imprinting has been associated to some diseases such as the Prader-Willi Syndrome or the Angelman Syndrome (Clayton-Smith [2003]).

The imprinted genes are typically clustered – as it is well known – in the so-called imprinting regions, rich in VNTRs (Constancia *et al*. [1998]). Moreover usually imprinting genes are flanked by VNTRs rich in C and G.

The imprinting is generally considered due to methylation (Constancia *et al*. [1998]), at the typical methylation site (the CG pair); Neumann (Neumann *et al*. [1995]) first suggested that such methylations are only a form of defence against virus insertions. Anyway the imprinting mechanisms are still unclear and some other hypotheses have been proposed (De Fonzo *et al*. [2000], Burns *et al*. [2001]). An important phenomenon that well deserves the name of imprinting is the fact that, in diseases due to triplets expansion, the intergenerational trend of VNTR length usually depends on maternal or paternal origin, even in the case of triplets where the CG pair is absent.

We quote only briefly the intriguing phenomena often called adaptive mutations, defined by some authors as genetic variations generated in response to the environment, rather than independently of it, as the neo-Darwinian theory would require. For a good review see Rosenberg [2001]. The very existence of phenomena of this kind is still highly controversial, but it is obvious that, if confirmed, they could be good examples of dynamical genetics.

The horizontal or lateral gene transfer is a phenomenon for which DNA fragments are transferred from an organism to another and are successively included in the recipient genome (Bushman [2002]). This process is in contrast to the inheritance of the genes, termed vertical transfer.

Horizontal transfer is very frequent in bacteria (Gogarten *et al*. [2002]):

- firstly bacteria are able to uptake tracts of the genome of dead bacteria (a phenomenon discovered by Griffiths [1928] and called transformation); only in 1944 Avery et al. [1944] discovered that such gene transmission was allowed by a DNA uptake;

- secondly among bacteria DNA transfer occurs directly from a cell (male) to another (female), via a process called conjugation (Lederberg and Tatum [1946]);
- thirdly DNA transfer occurs indirectly, via virus infection, by a process called transduction (Zinder and Lederberg [1952]).

In eukaryotes, in general the relevance of the horizontal transfer phenomenon is controversial (besides being a very hot subject because its frequent involvement in GMO quarrels), while it is undisputed in some cases in protozoa (Deitsch et al. [2001]).

The mammalian genome contains a small number of genes for which the foreign origin is generally accepted, although a complete cycle, from the DNA uptake to the inheritance of the acquired genes, has not yet been observed. There are however some experimental evidences of single steps of the complete cycle; we note for example that:

- there exist experimental evidences of mechanisms for active transport of long undegraded DNA tracts from the gastrointestinal tract to the bloodstream (Schubbert *et al*. [1994]) and then to the nucleus of some cells (Schubbert *et al*. [1997]). Further experiments on pregnant mice show that the placental transmission of such foreign DNA to foetus cells is rare but possible (Schubbert *et al*. [1998]);
- there exist, on cell membrane, suitable, partially unknown, receptors (Siess *et al*. [2000]) apt to cause the entering of nucleic acids (via endocytosis);
- the translin (the Testis-Brain RNA-Binding Protein in mice) is involved in recombination and translocation events, and appears to transport nucleic acids from cell to cell in testes or along axons in the brain (Wu *et al*. [1999a], Wu *et al*. [1999b], Hecht [2000], Pascal *et al*. [2001], Morales *et al*. [2002]) and could be involved in the hypothetical DNA managements occurring for memorisation (see section 2);
- the vimentin fibres (Hartig *et al*. [1998]) rapidly transport into the nucleus some of the DNA fragments that entered the cell. We deem important to observe that such DNA fragments, being rich in G, are just those that, in our opinion, are prone to form quadruplexes;
- the oligonucleotide quadruplexes exhibit a pharmacological (e.g. antiproliferative) activity (see section 5) once entered the nucleus, although they were added outside the cell.

As for the question about if and how such foreign DNA is transmitted to offspring, usually, as far as we know, experiments focus on pregnant females and not on males, although, as seen above, there exist in the testes mechanisms that in our opinion are quite likely occasionally apt to include in the genome some foreign DNA.

7. ON THE DYNAMICAL MECHANISMS IN CREUTZFELDT-JAKOB DISEASE.

The Mad Cow Disease (Bovine Spongiform Encephalopathy, BSE), the Sheep Scrapie and their human versions (e.g. Creutzfeldt-Jakob Disease, CJD) are examples of neurodegenerative diseases of a very peculiar nature: such diseases are the only genetic diseases that are also infectious, in that for example the ingestion of genetically sick brains can cause contagion. In the infectious case contagion is due to an amyloid structure, called prion, that accumulates inside the neurons causing their death.

Prions are composed mainly of a protein called Scrapie Prion Protein (PrP^{Sc}), a peculiar modification of the Cellular Prion Protein (PrP^{C}) normally present in neurons; a noteworthy characteristic of prions is that they are poor in nucleic acids.

CJD has many obscure, or at least disputed, aspects: the currently most accepted theory is due to Prusiner (Prusiner [1998]). According to this theory a sort of chain reaction

occurs, where infective transmission is caused just by PrP^{Sc}, which induces the prion protein to refold from the cellular to the scrapie form; and this without any contribution of nucleic acids, unlike all other infective diseases. The basis of this explanation is that in prions long tracts of nucleic acid seem to be absent (Oesch *et al*. [1988]) and the few tracts really found are considered too short to constitute a virus (Prusiner [1998]).

Although a number of experiments apparently support Prusiner model, other experiments hardly fit into this theory. A well known example is the existence of a number of different strains of the disease (Casalone *et al*. [2004]). Therefore other theories have been proposed, mostly based on nucleic acids: for example the virino theory (Liberski and Jaskolski [2002]) and the nemavirus theory (Narang [2001]). For a good review papers see Chesebro [2003] and Aguzzi and Polymenidou [2004].

Within the simple Prusiner framework the prion protein would find no place in a survey about dynamical genetics. There is however a growing body of experimental evidence suggesting that the situation is rather more complicated, and exhibits interesting similarities with many of the dynamic mechanisms considered above. Some examples follow.

- Apart from the fact that the normal cellular function of PrP remains elusive, there exist evidences showing that PrP^{C} is involved in a number of diverse phenomena (Derrington and Darlix [2002]), as often occurs in TRLMs, and unlike most proteins involved in simple metabolic processes (see section 1).
- In some hereditary forms of CJD there is, in the encoding region of the prion gene, a polymorphism of a 24-nucleotide VNTR (that appears to be stable along the germline), the so-called octapeptide repeat: a number of repeats greater than the normal value yields the disease (Goldfarb *et al*. [1991]). We note that this is what happens in many VNTR diseases (as for Huntington, see above), and that a number of such diseases, like CJD, also exhibit late onset and are neurodegenerative.
- There exist many experimental results showing unexpected relationships between PrP and nucleic acids. Some relationships can be found in a recent annotated survey (Web page of Jean Marc Gabriel http://www.pub-internet.com/ESBPRIONhiv.htm) of ten papers about common features of HIV and CJD; we can add Scheffer *et al*. [1995], Mahfoud *et al*. [2002], and Barrette *et al*. [2001]. The last reference reports computer studies that suggest a peculiar ("pseudoknot") 3D structure of the PrP mRNA located just at the VNTR site; and hypothesises an involvement with CJD of such a structure, similar to the 3D structure of a tract of the HIV RNA.
- Many other experiments show that in some cases PrP and nucleic acids exhibit a mutual influence (a usual fact for viral proteins, as those of HIV, but a very rare fact for cellular proteins). For example PrP acts in various ways on nucleic acids inducing for example folding or aggregation (Gabus *et al*. [2001], Derrington *et al*. [2002], Nandi and Sizaret [2001], Moscardini *et al*. [2002]) and nucleic acids acts in various ways on PrP: Deleault *et al*. [2003] show that in vitro specific RNA molecules are required for prion protein conversion; Cordeiro *et al*. [2004] state that DNA modifies PrP; Nandi *et al*. [2002] state that DNA induces polymerisation.

Another theory, alternative to Prusiner theory, has been proposed, which is also based on nucleic acids, and is more explicitly inspired to dynamical genetics (De Fonzo *et al*. [2000]). According to this theory the contagion would be due to some oligonucleotides (especially those corresponding to the octapeptide repeat) that are carried by the prion.

Within this framework the physiological function of the PrP in the brain would be inside a mechanism for the management of nucleic acids, and the different forms of PrP would be used to transport, to uptake, or to store RNA or even DNA, and this would well fit in the context of theories about long-term memory based on nucleic acids, described in section 2; and the failure of some of these physiological mechanisms would cause the disease.

An important support is provided by recent works showing an implication of PrP in long-term memory (Wickelgren [2004] and references therein).

We note that this framework would naturally explain most of the above surprising experimental results, and in particular the results found after than the above theory was proposed could be well considered a good confirmation of the theory.

Moreover, a subsequent paper (De Fonzo *et al*. [2001]) suggests that the above oligonucleotides (those corresponding to the VNTR) could well form quadruplex structures, and this, beside possible useful functions, could explain the outstanding stability of the infectious agent. Two interesting, and somehow related, experimental results provide some indirect plausibility to such hypothesis:

- in some cases for the interaction between nucleic acids and prion protein a quadruplex structure formation is mandatory (Weiss *et al*. [1997]);
- the optimal UV wavelength to inactivate the prions differs from the typical wavelength used to damage nucleic acids (Alper *et al*. [1978]). Although this is usually considered an important argument against nucleic acids theories, such argument neglects the fact that the inactivation and the absorption spectra of nucleic acids are context- and conformation-dependent: for example Chesebro [2003] points out that the inactivation spectrum of the RNA of Tobacco Mosaic Virus is similar to the prion's spectrum when the virus is intact (and differs from the inactivation spectrum of naked RNA), with its RNA packed inside its protein envelope. Moreover, since the quadruplexes have a spectral behaviour different from the double helix (Mergny and Helene [1998]), the fact that the contagious agents were the quadruplexes would be even less surprising.

8. CONCLUDING REMARKS

We examined here a number of phenomena of growing biological importance that have no explanation within a static approach to genetics, but are well treated in a new dynamical approach to genetics that may facilitate their interpretation.

This newly born dynamical genetics is both too young and already too crowded to prevent an exhaustive survey, especially within the space limitations of a paper: we rather chose to perform a tentative survey, emphasising only the more intriguing aspects, taking full responsibility of our choices, and apologising for the unavoidable unbalances and omissions.

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UN PANORAMA DELLA GENETICA DINAMICA

Riassunto

La genetica "classica" è basata sul dogma centrale della biologia molecolare che assegna al DNA un ruolo fondamentale ma statico. Secondo questo dogma, il DNA si può duplicare solo in copie identiche (salvo che per errori aleatori), e nessun meccanismo "intelligente" può alterarne il contenuto informativo: in maggiore dettaglio l'informazione genetica fluisce solo dal DNA tramite l'RNA alle proteine e mai viceversa.

Tuttavia, dal cosiddetto genoma dinamico (i "jumping genes" di Barbara McClintock) alle cosiddette mutazioni dinamiche (quali ad esempio l'espansione di trinucleotidi), si sta scoprendo un numero crescente di casi importanti in cui il DNA è alterato in modo più o meno sofisticato, spesso da meccanismi enzimatici intelligenti. Lo studio di tutti questi fenomeni dinamici e delle loro interpretazioni può ben essere chiamato genetica dinamica.

Fra i fenomeni coinvolti nel comportamento dinamico del DNA, di crescente importanza sta diventando la presenza dei cosiddetti quadruplexes. Un quadruplex è una peculiare struttura del DNA (non a doppia elica), composta da quattro filamenti di DNA, stabilizzata da qualche quartetto di basi disposte in quadrato. I casi più frequenti ed interessanti (e anche i più stabili termodinamicamente) si hanno quando le quattro basi sono guanine, e in questo caso si parla di quartetti G.

Un'altro fenomeno importante nel comportamento dinamico del DNA è la presenza di VNTR, ripetizioni consecutive (TR, Tandem Repeats), in numero variabile, di sequenze di trinucleotidi o polinucleotidi; l'importanza dei VNTR è ormai indiscussa anche per la loro connessione con numerose malattie, spesso neurodegenerative.

Nel presente articolo esaminiamo un certo numero di fenomeni dinamici ed anche alcuni di grande importanza biologica che non hanno una spiegazione universalmente accettata all'interno di un approccio statico alla genetica, ma per i quali un'interpretazione dinamica è stata solo proposta, per esempio fenomeni controversi ma interessanti, quali la trasmissione orizzontale e il morbo di Creutzfeldt-Jacob.