DeepG4 : A deep learning approach to predict active G-quadruplexes

Vincent Rocher[1*], Matthieu Genais[1*], Elissar Nassereddine[1] and Raphael Mourad[1+]

July 23, 2020

[1] Laboratoire de Biologie Cellulaire et Moléculaire du Contrôle de la Prolifération (LBCMCP), CNRS, Université Paul Sabatier (UPS), 31000 Toulouse, France

*Contributed equally.
+Corresponding author: raphael.mourad@univ-tlse3.fr.

Abstract

DNA is a complex molecule carrying the instructions an organism needs to develop, live and reproduce. In 1953, Watson and Crick discovered that DNA is composed of two chains forming a double-helix. Later on, other structures of DNA were discovered and shown to play important roles in the cell, in particular G-quadruplex (G4). Following genome sequencing, several bioinformatic algorithms were developed to map G4s in vitro based on a canonical sequence motif, G-richness and G-skewness or alternatively sequence features including k-mers, and more recently deep learning. Here, we propose a novel convolutional neural network (DeepG4) to map active G4s (forming both in vitro and in vivo). DeepG4 is very accurate to predict active G4s, while most state-of-the-art algorithms fail. Moreover, DeepG4 identifies key DNA motifs that are predictive of G4 activity. We found that active G4 motifs do not follow a very flexible sequence pattern as current algorithms seek for. Instead, active G4s are determined by numerous specific motifs. Moreover, among those motifs, we identified known transcription factors which could play important roles in G4 activity by contributing either directly to G4 structures themselves or indirectly by participating in G4 formation in the vicinity. Lastly, variant analysis suggests that SNPs altering predicted G4 activity could affect transcription and chromatin, *e.g.* gene expression, H3K4me3 mark and DNA methylation. Thus, DeepG4 paves the way for future studies assessing the impact of known disease-associated variants on DNA secondary structure by providing a mechanistic interpretation of SNP impact on transcription and chromatin.

1 Introduction

Deoxyribonucleic acid (DNA) is a complex molecule carrying genetic instructions for the development, functioning, growth and reproduction of all known living beings and numerous viruses. In 1953, Watson and Crick discovered that DNA is composed of two chains forming a double-helix [26]. However, other structures of DNA were discovered later and shown to play important roles in the cell. Among those structures, G-quadruplex (G4) was discovered in the late 80's [23]. G4 sequence contains four continuous stretches of guanines (G-tracts). Four guanines can be held together by Hoogsteen hydrogen bonding to form a square planar structure called a guanine tetrad (G-quartets). Two or more G-quartets can stack to form a G4. The quadruplex structure is further stabilized by the presence of a cation, especially

potassium, which sits in a central channel between each pair of tetrads.

G4s were found enriched in gene promoters, DNA replication origins and telomeric sequences. Accordingly, numerous works suggest that non-B DNA structures can regulate several essential processes in the cell, such as gene transcription, DNA replication, DNA repair, telomere stability and V(D)J recombination [24]. For instance, telomeres are sequences consisting of 300-8000 precise repeats of TTAGGG. They can form G4 structures that inhibit telomerase activity responsible for maintaining length of telomeres and are associated with most cancers [25]. G4s can also regulate gene expression such as for MYC oncogene where inhibition of the activity of NM23-H2 molecules that bind to the G4 silences gene expression [5]. Moreover, G4s are also fragile sites and prone to DNA double-strand breaks [19]. Accordingly, G4s are highly suspected to be implicated in human diseases such as cancer or neurological/psychiatric disorders [1, 7].

Following the Human Genome project, computational algorithms were developed to predict the location of G4 sequence motifs in the human genome [14, 15]. Such algorithms consisted in finding all occurrences of the canonical motif $G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$ (or the corresponding C-rich motif). Using this canonical motif, over 370 thousand G4s were found in the human genome. Nonetheless, such pattern matching algorithms lacked flexibility to accomodate for possible divergences from the canonical pattern. To tackle this issue, novel score-based approaches were developed to compute G4 propensity score by quantifying G-richness and G-skewness [4], or by summing the binding affinities of smaller regions within the G4 and penalizing with the destabilizing effect of loops [12]. A machine learning approach was proposed to predict G4s based on sequence features (such as k-mer occurrences) and trained using newly available genome-wide mapping of G4s in vitro named G4-seq [22]. Deep learning was also recently developed to predict G4s. PENGUINN, a deep convolutional neural network (CNN), was trained to predict G4s in vitro [16]. Another CNN, G4detector, was designed to predict G4s forming both in vitro and in vivo [3]. Thus, all current approaches, except G4detector, aimed to predict G4s in vitro and but were not designed to assess the ability of G4 sequences to form in vivo (*e.g.* G4 activity).

Here, we propose a novel method, named DeepG4, aimed to predict G4 activity from DNA sequence. DeepG4 implements a CNN which is trained using a combination of available in vitro (G4-seq) and in vivo (BG4 ChIP-seq) genome-wide mapping data. DeepG4 achieves excellent accuracy (area under the receiver operating characteristic curve or AUROC > 0.956). DeepG4 outperforms by far all existing non-deep learning algorithms including G4Hunter, pqsfinder and Quadron, and performs slightly better or similarly compared to existing deep learning approaches G4detector and PENGUINN. DeepG4 can predict well across cell lines. Moreover, DeepG4 identifies key DNA motifs that are predictive of active G4s. Among those motifs, we found specific motifs resembling the G4 canonical motif (or parts of G4 canonical motif), but also numerous known transcription factors which could play important roles in G4 activity directly or indirectly. In addition, analysis of publicly available SNPs revealed that SNPs increasing predicted G4 activity were associated with higher gene expression, higher H3K4me3 mark and lower DNA methylation.

2 Materials and Methods

2.1 BG4 ChIP-seq and G4-seq data

We downloaded BG4 ChIP-seq data for HaCaT, K562 and HEKnp cell lines from Gene Expression Omnibus (GEO) accession numbers GSE76688, GSE99205 and GSE107690. Data were mapped to hg19. We downloaded processed G4-seq peaks mapped to hg19 from GEO accession number GSE63874. We used G4-seq from the sodium (Na) and potassium (K) conditions.

2.2 Active G4 sequences

We defined positive DNA sequences (active G4 sequences) as having the propensity to form both in vitro and in vivo G4s as follows. We only kept BG4 ChIP-seq peaks overlapping with G4-seq peaks. We then used the 201-bp DNA sequences centered on the BG4 ChIP-seq peak summits.

As negative (control) sequences, we used sequences randomly drawn from the human genome with sizes, GC, and repeat contents similar to those of positive DNA sequences using genNullSeqs function from gkmSVM R package (https://cran.r-project.org/web/packages/gkmSVM).

2.3 G4 canonical motif detection

We scanned DNA sequences for G4 canonical motifs with pattern $G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$ (or the corresponding C-rich pattern) as implemented in Quadparser algorithm [14, 15].

2.4 G4Hunter and pqsfinder

We computed G4 propensity score using G4Hunter with default parameters: threshold = 1.5 and width = 25 [4]. We also computed G4 propensity score using pqsfinder with default parameters [12]. For a given sequence, several G4 motifs can be detected. In order to obtain only one score per sequence, we used the maximum score.

2.5 Quadron

We used machine learning approach Quadron to compute G4 propensity [22].

2.6 PENGUINN and G4detector

We used deep learning approaches PENGUINN and G4detector to compute G4 probability [3, 16]. For a fair comparison with DeepG4, PENGUINN and G4detector were retrained using Keras. For each model, the best AUC was picked among the epochs.

2.7 JASPAR DNA motifs

We used PWMs for transcription factor binding sites from the JASPAR 2018 database (http://jaspar.genereg.net). We assessed motif enrichment using AME program from MEME suite.

2.8 CTCF and SP1 ChIP-seq data

CTCF and SP1 ChIP-seq data for K562 cell line were downloaded from ENCODE (https://genome.ucsc.edu/ENCODE/).

2.9 SNP data

We downloaded SNPs associated with gene expression from GTEx project (https://www.gtexportal.org/home/datasets). We downloaded SNPs associated with chromatin modifications from Delaneau *et al.* [9]. We also downloaded SNPs associated with DNA methylation from Pancan-meQTL database [10].

2.10 DeepG4 model

DeepG4 is a feedforward neural network composed of several layers. DNA sequence is first encoded as a one-hot encoding layer. Then, a 1-dimension convolutional layer is used with kernels. A local average pooling layer is next used. Then, the global max pooling layer extracts the highest signal from the sequence. Dropout is used for regularization. A dense layer then combines the different kernels and the activation sigmoid layer allows to compute the probability of a sequence to be an active G4. The model is described in details in Subsection Results and Discussion, Deep learning approach.

Hyperparameters including the number of kernels (900), kernel size (20bp), kernel activation (relu), pool size (10bp), drop-out (0%), epoch number (20), number of neurons in the dense layer (100) and the optimizer choice (rmsprop) were found by fine-tuning with a random grid.

2.11 DNA motifs from DeepG4

The first layer of DeepG4 contained kernels capturing specific sequence patterns similar to DNA motifs. For a given kernel, we computed activation values for each positive sequence. If a positive sequence contained activation values above 0, we extracted the sub-sequence having the maximum activation value. The set of sub-sequences was then used to obtain a position frequency matrix (PFM) by computing the frequency of each DNA letter at each position for the kernel.

Each kernel PFM was then trimmed by removing low information content positions at each side of the PFM (threshold > 0.9). PFMs whose size were lower than 5 bases after trimming were removed. PWMs were next computed from PFMs assuming background probability of 0.25 for each DNA letter as done in JASPAR.

Because many PWMs from DeepG4 were redundant, we used the motif clustering program matrix-clustering from RSAT suite (http://rsat.sb-roscoff.fr/) with parameters: median, cor=0.6, ncor=0.6. We used PWM cluster centers as DNA motifs for further analyses.

2.12 Predicting SNP effect on G4 activity using DeepG4

We used DeepG4 to predict the effect of a SNP on G4 activity as follows. We computed a $\Delta score$ as the difference between the DeepG4 score of the sequence with the SNP (alternative allele) and the DeepG4 score of the sequence without the SNP (reference allele). In order to account for the SNP context, G4 activity was predicted from the 201-base sequence surrounding the SNP. Instead of using DeepG4 last layer output (a probability varying between 0 and 1) as a score, we used the output from the second to the last layer to obtain a score varying from $-\infty$ to $+\infty$, allowing a better measure for $\Delta score$.

2.13 DeepG4 implementation

DeepG4 was implemented using Keras R library (https://keras.rstudio.com/). DeepG4 is available at https://github.com/morphos30/DeepG4.

3 Results and Discussion

3.1 Deep learning approach

Our computational approach for predicting active G4 sequences is schematically illustrated in Figure 1. In the first step (Figure 1A), we retrieved recent genome-wide mapping of in vitro G4 human sequences

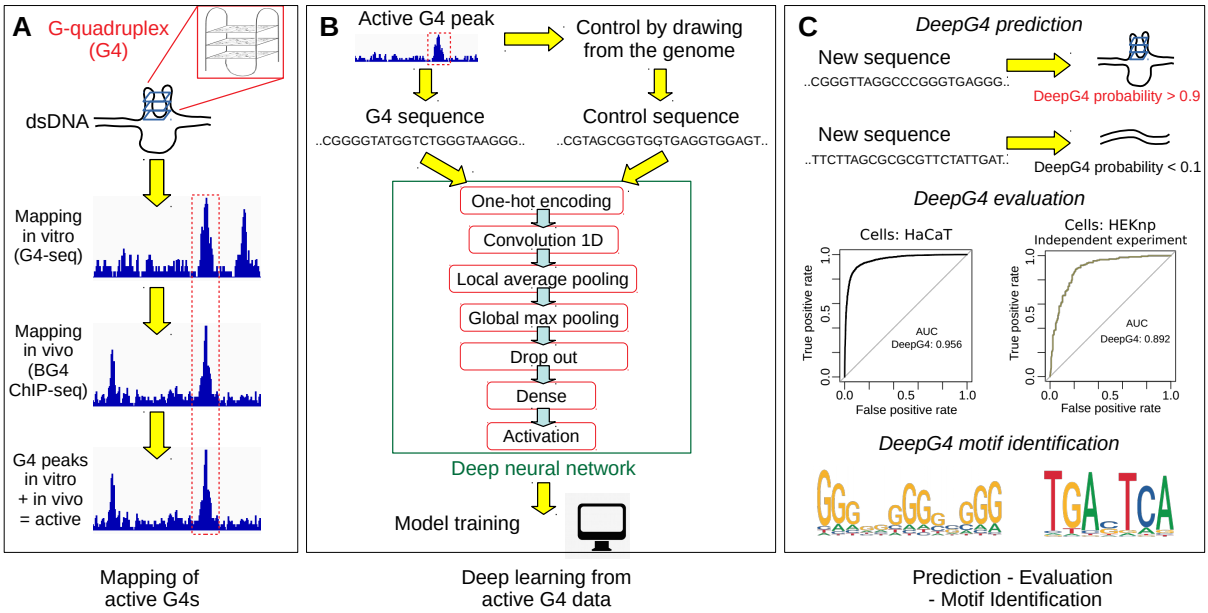

Figure 1. Illustration of DeepG4. A) Mapping of active G4 sequences both in vitro and in vivo using NGS techniques. B) Deep learning model training using active G4 and control sequences. C) G4 activity prediction, evaluation and motif identification.

using G4-seq data [6] and of in vivo G4 human sequences using BG4 ChIP-seq data (BG4 is an antibody binding G4s) [11]. Both methods mapped G4s at high resolution (few hundred base pairs). By overlapping BG4 ChIP-seq peaks with G4-seq peaks, we could identify a set of G4 peaks that were formed both in vitro and in vivo, and which we called BG4-G4-seq peaks and considered as "active G4s".

In the second step (Figure 1B), we extracted the DNA sequences from active G4 peaks (positive sequences). As negative sequences, we used sequences randomly drawn from the human genome with sizes, GC, and repeat contents similar to those of positive DNA sequences. Positive and negative sequences were then used to train our deep learning classifier called DeepG4. DeepG4 is a feedforward neural network composed of several layers. DNA sequence is first encoded as a one-hot encoding layer. Then, a 1-dimension convolutional layer is used with 900 kernels and a kernel size of 20 bp. A local average pooling layer with a pool size of 10 bp is next used. This layer is important: it allows to aggregate kernel signals that are contiguous along the sequence, such that a G4 sequence can be modeled as multiple contiguous small motifs containing stretches of Gs. For instance, a G4 sequence can be defined by two contiguous motifs GGGNNNGGG separated by 5 bases, yielding the canonical motif GGGNNNGGGNNNNNGGGNNNGGG. Then, the global max pooling layer extracts the highest signal from the sequence for each kernel. Dropout is used for regularization. A dense layer then combines the different kernels. The activation sigmoid layer allows to compute the probability (or score) of a sequence to be an active G4.

In the third step (Figure 1C), we used DeepG4 to predict the G4 activity (probability between 0 and 1) for a novel DNA sequence. We split the sequence data into a training set to learn model parameters, a validation set to optimize hyper-parameters and a testing set to assess model prediction accuracy. For this purpose, we computed the receiver operating characteristic (ROC) curve and the area under the ROC (AUROC). DeepG4 motifs are identified from the convolutional layer.

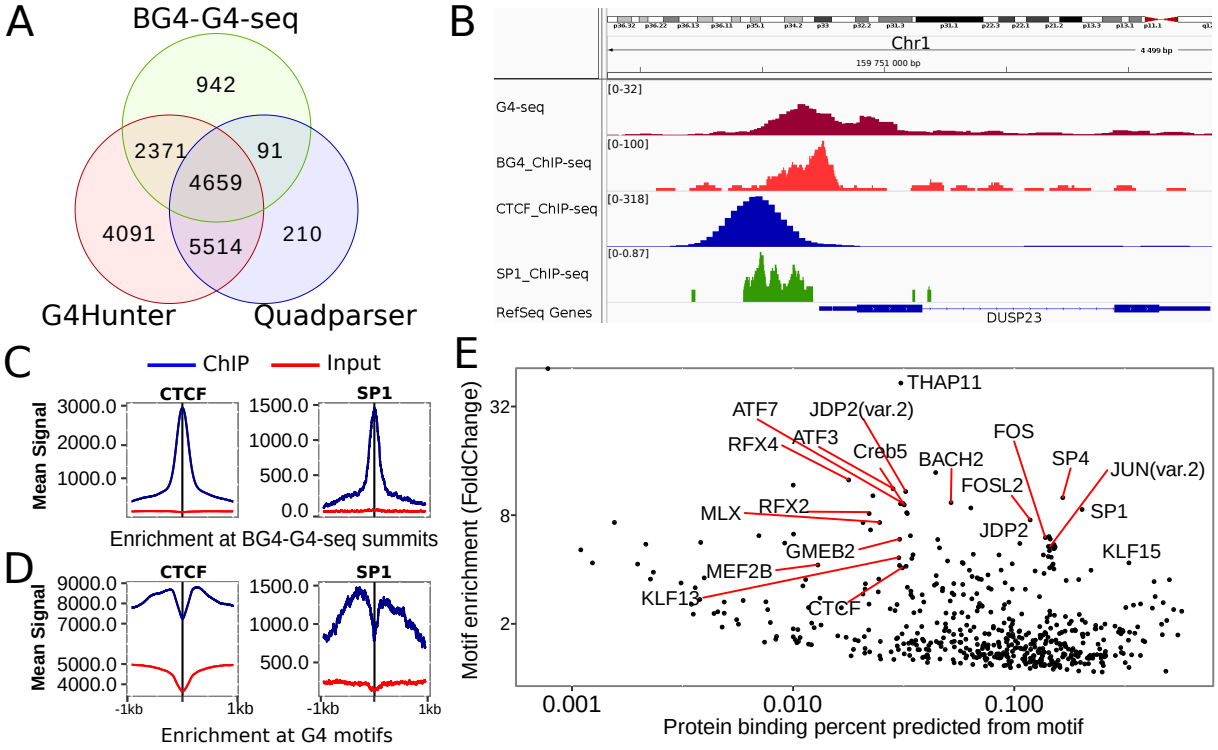

Figure 2. Sequence features enriched at G4s. A) Venn diagram showing the overlap between BG4-G4-seq peaks, Quadparser canonical motifs and G4Hunter predictions at promoter regions. B) Genome browser view of G4 mapping in vitro (G4-seq) and in vivo (BG4 ChIP-seq) around the promoter region of gene DUSP23. CTCF and SP1 ChIP-seq were also displayed. Data were from K562 cell line. C) CTCF and SP1 ChIP-seq signal averaged at BG4-G4-seq peak summits. D) CTCF and SP1 ChIP-seq signal averaged at G4 canonical motif sites. E) JASPAR motif enrichment at BG4-G4-seq peaks depending on the percentage of binding. Here we used BG4-G4-seq peaks from HaCaT cell line.

3.2 Sequence features enriched at active G4s

We first assessed the link between experimentally mapped G4s and known DNA sequence features. For this purpose, we looked at the overlap between active G4s (BG4-G4-seq) peaks and predicted G4s from Quadparser and G4Hunter algorithms at gene promoters (Figure 2A). We found an important overlap between experimental and predicted G4s, *e.g.* 7118/8060=88% of BG4-G4-seq peaks overlapped any predicted G4s. However, this results also showed that a non-negligible fraction of BG4-G4-seq peaks (11%) could not be predicted by any of the two algorithms. Moreover, numerous predicted G4s by Quadparser (210) and by G4Hunter (4091) were not detected experimentally as active G4s. This result motivated us to seek for other DNA sequence features that could help to better predict active G4s.

Known transcription factor binding site (TFBS) motifs are good candidates, since G4s are known to be strongly enriched at promoters. TFs are thus expected to bind in the vicinity of G4s or potentially at G4s themselves. For instance, by browsing G4 data together with other TF data, we observed that active G4s (BG4 ChIP-seq and G4-seq) colocalized with CTCF and SP1 ChIP-seq peaks (Figure 2B). More globally, CTCF and SP1 signals were strongly enriched at BG4-G4-seq peak summits (CTCF: FC=10.5; SP1: FC=55.3; Figure 2C). Similarly, we found strong enrichments of CTCF and SP1 signals

at promoter canonical G4 motifs (CTCF: FC=31.3; SP1: FC=47.9; Figure 2D). In agreement, it was previously found that SP1 binds to G4s with a comparable affinity as its canonical motif [21]. Recently, G4s were found enriched at 3D domain (topologically associating domain, TAD) borders in the vicinity of the architectural protein CTCF, suggesting a role for G4s in 3D genome architecture [13].

We then comprehensively explored TF binding at BG4-G4-seq peaks by analyzing the enrichment of all known TF DNA motifs from JASPAR database using MEME AME program [20] (Figure 2E). Interestingly, we found that numerous TF motifs were enriched at active G4s, in addition to CTCF and SP1. For instance, we found THAP11 (MA1573.1), a repressor that plays a central role for embryogenesis and the pluripotency of embryonic stem (ES) cells. Moreover, we found TF members of the AP-1 complex, *e.g.* FOS and JUN, which regulates gene expression in response to a variety of stimuli. We also found KLF factors that belonged to the same TF family as SP factors. Thus, these observations suggested that the presence of specific TFs could contribute to the formation of active G4s in addition to the presence of the G4 canonical motif.

3.3 G4 predictions with DeepG4

We developed a deep learning model, called DeepG4, to predict active G4s (BG4-G4-seq peaks). DeepG4 is a classifier that discriminates between G4 and control sequences. We split the sequences into a training set to learn model parameters, a validation set for hyperparameter optimization and a testing set to evaluate prediction accuracy. As control sequences, we randomly selected genomic sequences that matched sizes, GC, and repeat contents of BG4-G4-seq peaks using R package gkmSVM (a standard way to obtain controls). Using DeepG4, we obtained excellent predictions of active G4s from HaCaT cells (Figure 3A; AUROC = 0.956; 95% confidence interval of AUROC: $[0.953, 0.960]$).

We then compared with state-of-the-art G4 prediction algorithms, which were previously shown to perform very well for in vitro G4 predictions. When assessed on active G4s, score-based algorithms G4Hunter and pqsfinder performed poorly (G4Hunter AUROC = 0.54, CI95% : $[0.532, 0.542]$; pqsfinder AUROC = 0.71, CI95% : $[0.702, 0.711]$; Figure 3B). G4Hunter required a threshold parameter which might not be properly set for active G4s (set to 1.5 as in [4]). Hence, we decided to implement G4Hunter within a machine learning approach, such that G4Hunter threshold parameter could be learned from data, thereby allowing a fairer comparison with DeepG4. For this purpose, we ran G4Hunter predictions for varying G4Hunter threshold values (1 to 2 by 0.1) as features for a random forest classifier (we named G4HunterRF). G4HunterRF strongly outperformed previous G4Hunter predictions (AUROC=0.81, CI95% : $[0.801, 0.814]$; Figure 3B), but the predictions were still less good than those from DeepG4. We then evaluated machine-learning approach Quadron that was originally trained from in vitro G4s. We found low prediction performance for Quadron (AUROC=0.58, CI95% : $[0.576, 0.583]$; Figure 3C). For a fairer comparison of Quadron with DeepG4, we decided to re-train Quadron using active G4 sequences (we named QuadronRetrained). Re-trained Quadron yielded greatly improved results (AUC=0.7, CI95% : $[0.690, 0.705]$; Figure 3C), but which were still far less accurate than those from DeepG4. CNN algorithm PENGUINN performed poorly (AUC=0.56, CI95% : $[0.559, 0.569]$; Figure 3D), but achieved very good performance when retrained (AUC=0.942, CI95% : $[0.938, 0.946]$; Figure 3D). G4detector, which was originally trained on both in vivo and in vitro data, achieved a good performance (AUC=0.77, CI95% : $[0.766, 0.774]$; Figure 3E), and very good performance when retrained (AUC=0.91, CI95% : $[0.902, 0.913]$; Figure 3E).

We next assessed prediction performance of DeepG4 on an independent dataset with the same cell line (HaCaT). DeepG4 showed very high accuracy with the independent dataset (AUC=0.95, CI95% : $[0.946, 0.954]$; Figure 3F). We then evaluated the ability of DeepG4 trained on one cell line (HaCaT) to predict G4s in another cell line. We found that DeepG4 could predict reasonably well active G4s

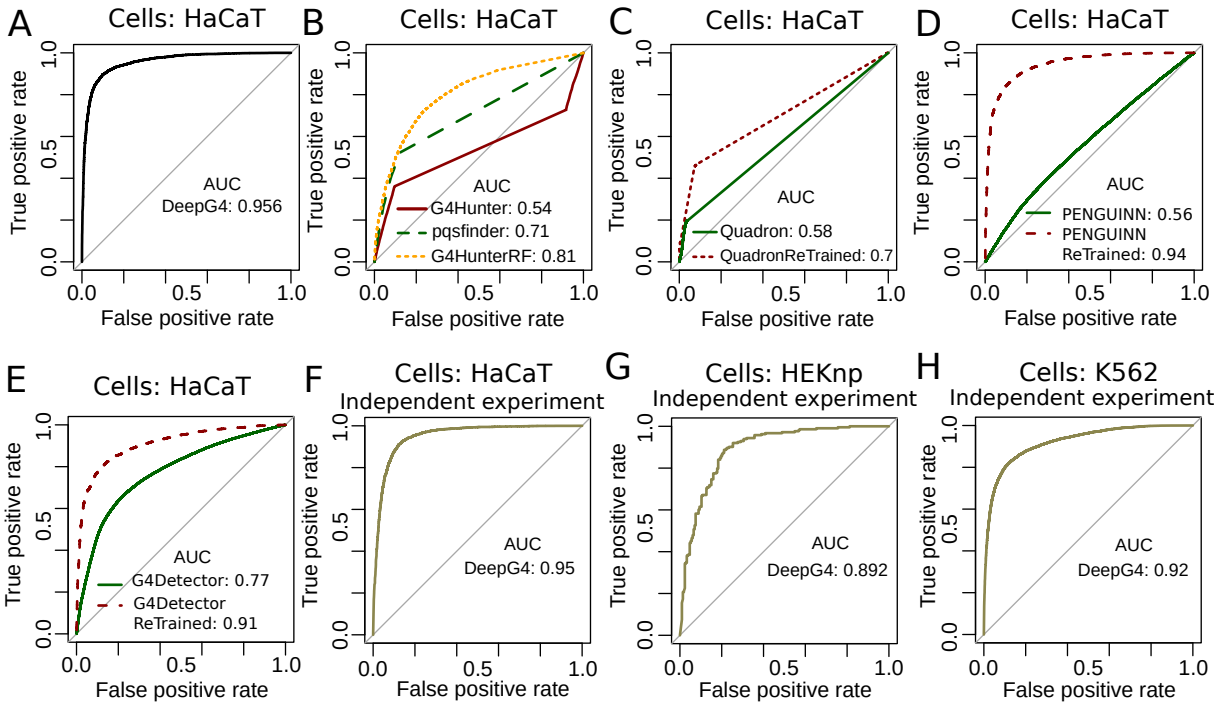

Figure 3. Prediction accuracy of DeepG4 and comparison with state-of-the-art algorithms. A) Prediction accuracy of DeepG4. The model was trained and evaluated using HaCaT cell data. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUROC) were plotted. B) Prediction accuracy of score-based algorithms G4Hunter, pqsfinder and G4HunterRF evaluated using HaCaT cell data. C) Prediction accuracy of machine-learning based algorithms Quadron and QuadronRetrained evaluated using HaCaT cell data. D) Prediction accuracy of deep-learning based algorithms Penguinn and PenguinnRetrained evaluated using HaCaT cell data. E) Prediction accuracy of deep-learning based algorithms G4Detector and G4DetectorRetrained evaluated using HaCaT cell data. F) Prediction accurary of DeepG4 trained using one experiment (GEO dataset GSE76688) and evaluated using another independent experiment (GEO dataset GSE99205) in HaCaT cells. G) Prediction accurary of DeepG4 trained using HaCaT data and evaluated in another cell line, HEKnp. H) Prediction accurary of DeepG4 trained using HaCaT data and evaluated in another cell line, K562.

in HEKnp cell line [11] (AUC=0.892, CI95% : $[0.861, 0.923]$; Figure 3G) and in K562 cell line too [18] (AUC=0.92, CI95% : $[0.917, 0.924]$; Figure 3H).

These results thus demonstrated the strong ability of DeepG4 to accurately predict active G4s from DNA sequences, and the slightly better or similar performances compared to retrained existing deep learning approaches. Conversely, results also showed the incapacity of state-of-the-art G4 non-deep learning algorithms to predict precisely G4s that were formed in vivo. Moreover, we showed that DeepG4 could accurately predict G4s in other cell lines.

3.4 Identification of important motifs from DeepG4

The first layer of DeepG4 convolutional neural network comprised kernels that encoded DNA motifs predictive of active G4s. Hence, we extracted from the first layer the kernels and converted them to DNA

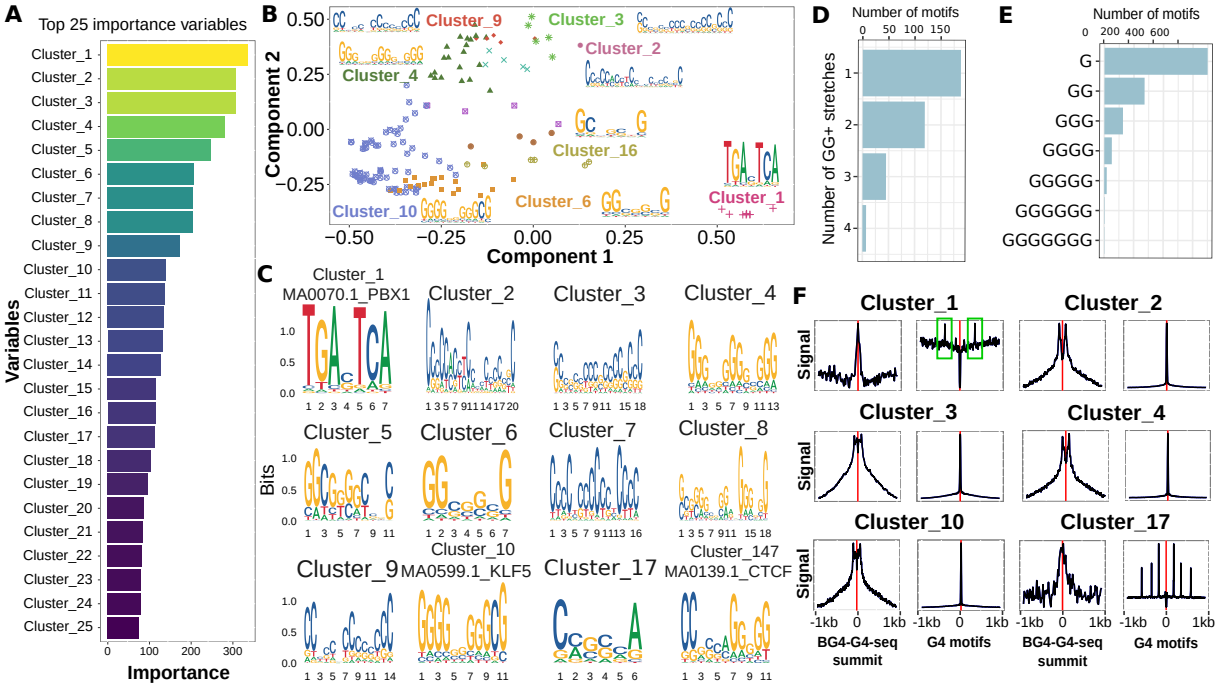

Figure 4. DNA motifs identified by DeepG4. A) Variable importances of DeepG4 cluster motifs, as estimated by random forests. Clustering of DeepG4 kernel motifs was done by RSAT matrix-clustering program to obtain cluster motifs. B) Multidimensional scaling (MDS) of DeepG4 motifs. As an input, matrix-clustering correlation matrix between kernel motifs was used. C) Logos of cluster motifs with highest variable importances. D) Number of kernel motifs containing one or more GG+ stretches. A GG+ stretch is defined as a stretch of 2 or more Gs in the motif consensus sequence. E) Number of kernel motifs containing G stretches depending on stretch length. F) Average profiles measuring the enrichment of cluster motifs centered around BG4-G4-seq summits or canonical G4 motifs.

motif PWMs to better understand which motifs were the best predictors of G4 activity. DeepG4 identified 900 motifs, many of them were redundant. To remove redundancy, we clustered the motifs using RSAT matrix-clustering program and kept the cluster motifs (also called root motifs in the program) for subsequent analyses. Cluster motifs could be divided into two groups: a group of de novo motifs and a group of motifs that resembled known TFBS motifs. To distinguish between these two groups, we used TomTom program (MEME suite) which mapped the cluster motifs to JASPAR database. DeepG4 motifs matching JASPAR were considered as known TFBS motifs, while motifs that did not match were classified as de novo motifs.

We then sought to assess the ability of DeepG4 motifs to predict G4s. Hence, we computed DeepG4 cluster motif variable importances using random forests and found strong predictors (Figure 4A). In order to visualize the cluster motifs on a map, we used multi-dimensional scaling (MDS), where we also added the original kernel motifs used to build the cluster motifs. We found that the first MDS component reflected the motif GC content (higher at the left side), while the second component represented the guanine stretch length (higher at the top) (Figure 4B).

Many strong predictors were de novo motifs which ressembled parts of G4 canonical motifs. For instance, cluster 4 comprised 3 stretches of GGG+, and could thus be considered as 3/4 of a canonical G4

motif (Figure 4C). Cluster 5 comprised two stretches of GG+, thus forming half of a canonical G4 motif. We then counted GG+ stretches (stretches of 2 or more guanines) from the kernel motifs and found that many kernel motifs contained more than one GG+ stretch (Figure 4D). Moreover, the guanine stretches were of vary lengths, ranging from one G up to 5 Gs (Figure 4E).

Among the best predictors, we also found several motifs corresponding to known TFBS motifs (Figure 4C). For instance, the best predictor, cluster 1, almost perfectly matched PBX1 motif MA0070.1 (q-value=0.011). Another strong predictor, cluster 10, matched KLF5 motif MA0599.1 (q-value=0.016). We could also identify a predictor, cluster 147, that matched well CTCF motif (q-value=0.0001), but whose variable importance was moderate.

We then assessed the enrichment of DeepG4 cluster motifs around BG4-G4-seq summits and around canonical G4 motifs. Motifs ressembling parts of G4 canonical motifs, such as clusters 2, 3 and 4, were enriched at both BG4-G4-seq summits and canonical G4 motif centers, thus representing actual G4 structures. But other motifs that were very different from the G4 canonical motif, such as cluster 1, were strongly enriched at BG4-G4-seq summits, but depleted at the exact location of canonical G4 motifs. Interestingly, cluster 1 was enriched close to canonical G4 motifs (around 300 bp, highlighted in green), suggesting that cluster 1 (PBX1 motif MA0070.1) did not participate directly to the G4 structure, but could act in the vicinity to support G4 activity. Similarly, cluster 17 motif, which did not resemble G4 parts nor matched a TF, showed enrichment at BG4-G4-seq summits, but was enriched in the vicinity of canonical G4 motifs. Moreover, we could consider a third class of cluster motifs, such as cluster 10, which matched a TF (KLF5) and was found strongly enriched at BG4-G4-seq summits and at the exact location of canonical G4 motifs.

These observations revealed the important role of TFBS motifs that could act directly in G4 activity as part of G4 structure, as previously shown for SP1 in vitro [21], or could participate indirectly to support G4 activity in the vicinity of G4s.

3.5 SNP effect on g-quadruplex

We then sought to explore the possible effect of SNPs on G4 activity using DeepG4, as a previous study showed that SNPs within G4 canonical motifs could affect gene expression [2]. For this purpose, we predicted G4 activity from the 201-base sequence surrounding a SNP. To assess the effect of a SNP, we computed a delta score as the difference between the DeepG4 score of the sequence with the SNP (alternative allele) and the DeepG4 score of the sequence without the SNP (reference allele).

We first analyzed the link between delta score and gene expression, and found that SNPs increasing gene expression presented higher G4 activity as compared to SNPs diminishing gene expression ($p = 9 \times 10^{-15}$; Figure 5A). We then explored the link between G4 activity and chromatin mark H3K4me3 which was often associated with the activation of transcription of nearby genes. We found that SNPs increasing H3K4me3 mark were associated with higher delta score as compared to SNP diminishing gene expression ($p = 3 \times 10^{-5}$; Figure 5B). Next, we looked at DNA methylation which is often involved in the inhibition of nearby genes. Moreover, G4 structures are known to affect DNA methylation [18]. In agreement, we observed that SNPs reducing DNA methylation showed higher G4 activity compared to SNPs increasing DNA methylation ($p = 3 \times 10^{-5}$; Figure 5C).

We next illustrated SNP effects on G4s with 2 examples. The SNP rs3748393 had a C allele as reference and A allele as alternative, and was associated with C16Orf95 gene repression in testis (gene expression regression $\hat{\beta} = -0.20$). Using DeepG4, we found that alternative allele A led to a decrease in G4 activity (Figure 5D). In fact, the SNP was part of a G4 motif composed of several G stretches

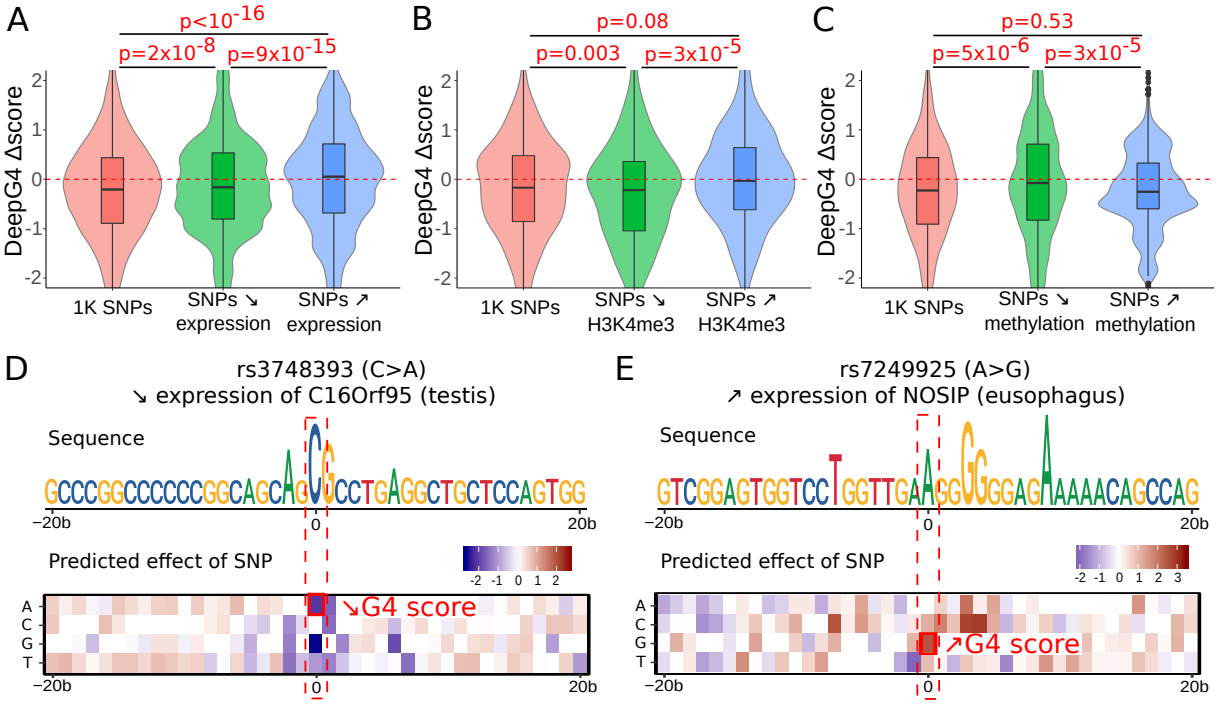

Figure 5. G-quadruplex SNPs are associated with gene expression and chromatin activity. A) SNP effect of g-quadruplex (delta score) depending on SNP effect on gene expression (regression beta). SNPs are from GTEx database [8]. SNPs from 1000 genomes project (1K SNPs) were added as controls. B) SNP effect of g-quadruplex (delta score) depending on SNP effect on H3K4me3 mark (regression beta). SNPs are from Delaneau *et al.* [9]. C) SNP effect of g-quadruplex (delta score) depending on SNP effect on DNA methylation (regression beta). SNPs are from Pancan-meQTL database [10]. D) Example of the SNP rs3748393 that is associated with gene expression decrease and which is predicted to decrease G4 activity. The predicted effect of SNPs for each allele and each position around rs3748393 is plotted as heatmap. E) Example of the SNP rs7249925 that is associated with gene expression increase and which is predicted to increase G4 activity.

(C stretches in the figure). Thus, by changing the C allele to A (corresponding to G>T on the reverse strand), the SNP could alter the G4 structure stability. As a second example, we then analyzed SNP rs7249925 that was associated with NOSIP gene induction in eusophagus (gene expression regression $\hat{\beta} = 0.16$). We found that the alternative allele G, which expanded a GGGGGG stretch, led to an increase of G4 activity (Figure 5E).

4 Conclusion

In this article, we propose a novel deep learning method, named DeepG4, to predict active G4s from DNA sequences. The proposed method is designed to predict G4s that are found both in vitro and in vivo, unlike previous algorithms that were developed to predict G4s forming in vitro (naked DNA). DeepG4 outperforms all existing non-deep learning algorithms even when those algorithms (re-)trained using active G4 data. This is due to the DeepG4 convolutional layer that allows to learn predictive features (here DNA motifs) from the DNA sequence without prior knowledge. Compared to current deep learning algorithms, DeepG4 performs slightly better or similarly even after retraining the model.

DeepG4 uncovered numerous specific DNA motifs predictive of active G4s. Some motifs resembled the canonical G4 motif (quadparser) or parts of it. Interestingly, many other motifs matched known TFBS motifs including PBX1 motif MA0070.1, KLF5 motif MA0599.1 and CTCF motif MA0139.1, suggesting that they could contribute directly to G4 structures themselves or participate indirectly in G4 activity in the vicinity.

Analysis of SNP effects on predicted G4s suggests that SNPs altering G4 activity could affect transcription and chromatin, *e.g.* gene expression, H3K4me3 mark and DNA methylation. In particular, we show that SNPs decreasing G4 activity tend to inhibit gene expression and H3K4me3, and conversely to increase DNA methylation. Thus, DeepG4 paves the way for future studies assessing the impact of known disease-associated variants on DNA secondary structure and provides a mechanistic interpretation of SNP impact on transcription and chromatin.

There are several limitations of the proposed approach. First, the prediction accuracy of DeepG4 strongly depends on existing datasets that are limited, especially regarding in vivo mapping. Once more techniques for in vivo G4 mapping will be developped, DeepG4 will need to be retrained in order to improve prediction accuracy. Second, the proposed model is rather simple. Further improvements could be done by adding other layers to the networks such as LSTM, GRU, attention and dilated convolution layers. Third, DeepG4 is limited to predict active G4s but a similar approach could be used to predict any active non-B DNA structure using permanganate/S1 nuclease footprinting data [17]. Fourth, DeepG4 only uses DNA sequence as input and thus cannot be cell-type specific. Incorporating chromatin accessibility with DNA-seq (or ATAC-seq) as input to DeepG4 will allow the model predict cell-type specific G4s.

Funding

This work was supported by the University of Toulouse and the CNRS.

Authors' contributions

VR conceived and implemented the model, analyzed the SNPs and wrote the Github reposity. MG analyzed DNA motifs from DeepG4 and carried out complementary SNP analysis. EN generated control sequences and compared DeepG4 with existing algorithms. RM designed the project, conceived the model and wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors are grateful to Balasubramanian lab for data (University of Cambridge, UK). The authors are also thankful to Matthias Zytnicki for comments.

References

[1] Sefan Asamitsu, Masayuki Takeuchi, Susumu Ikenoshita, Yoshiki Imai, Hirohito Kashiwagi, and Norifumi Shioda. Perspectives for applying g-quadruplex structures in neurobiology and neuropharmacology. *International Journal of Molecular Sciences*, 20(12), 2019.

[2] Aradhita Baral, Pankaj Kumar, Rashi Halder, Prithvi Mani, Vinod Kumar Yadav, Ankita Singh, Swapan K. Das, and Shantanu Chowdhury. Quadruplex-single nucleotide polymorphisms (Quad-SNP) influence gene expression difference among individuals. *Nucleic Acids Research*, 40(9):3800–3811, January 2012.

[3] Mira Barshai and Yaron Orenstein. Predicting G-quadruplexes from DNA sequences using multi-kernel convolutional neural networks. In *Proceedings of the 10th ACM International Conference on Bioinformatics, Computational Biology and Health Informatics*, BCB 19, page 357365, New York, NY, USA, 2019. Association for Computing Machinery.

[4] Amina Bedrat, Laurent Lacroix, and Jean-Louis Mergny. Re-evaluation of G-quadruplex propensity with G4Hunter. *Nucleic Acids Research*, 44(4):1746–1759, 01 2016.

[5] Tracy A. Brooks and Laurence H. Hurley. Targeting MYC expression through G-quadruplexes. *Genes & Cancer*, 1(6):641–649, 2010. PMID: 21113409.

[6] Vicki S. Chambers, Giovanni Marsico, Jonathan M. Boutell, Marco Di Antonio, Geoffrey P. Smith, and Shankar Balasubramanian. High-throughput sequencing of DNA G-quadruplex structures in the human genome. *Nature Biotechnology*, 33(8):877–881, 2015.

[7] Graziella Cimino-Reale, Nadia Zaffaroni, and Marco Folini. Emerging role of G-quadruplex DNA as target in anticancer therapy. *Current Pharmaceutical Design*, 22(44):6612–6624, 2016.

[8] GTEx Consortium. Genetic effects on gene expression across human tissues. *Nature*, 550(7675):204–213, Oct 2017.

[9] O. Delaneau, M. Zazhytska, C. Borel, G. Giannuzzi, G. Rey, C. Howald, S. Kumar, H. Ongen, K. Popadin, D. Marbach, G. Ambrosini, D. Bielser, D. Hacker, L. Romano, P. Ribaux, M. Wiederkehr, E. Falconnet, P. Bucher, S. Bergmann, S. E. Antonarakis, A. Reymond, and E. T. Dermitzakis. Chromatin three-dimensional interactions mediate genetic effects on gene expression. *Science*, 364(6439), 2019.

[10] Jing Gong, Hao Wan, Shufang Mei, Hang Ruan, Zhao Zhang, Chunjie Liu, An-Yuan Guo, Lixia Diao, Xiaoping Miao, and Leng Han. Pancan-meQTL: a database to systematically evaluate the effects of genetic variants on methylation in human cancer. *Nucleic Acids Research*, 47(D1):D1066–D1072, September 2018.

[11] Robert Hänsel-Hertsch, Dario Beraldi, Stefanie V. Lensing, Giovanni Marsico, Katherine Zyner, Aled Parry, Marco Di Antonio, Jeremy Pike, Hiroshi Kimura, Masashi Narita, David Tannahill, and Shankar Balasubramanian. G-quadruplex structures mark human regulatory chromatin. *Nature Genetics*, 48(10):1267–1272, 2016.

[12] Ji Hon, Tom Martnek, Jaroslav Zendulka, and Matej Lexa. pqsfinder: an exhaustive and imperfection-tolerant search tool for potential quadruplex-forming sequences in R. *Bioinformatics*, 33(21):3373–3379, July 2017.

[13] Yue Hou, Fuyu Li, Rongxin Zhang, Sheng Li, Hongde Liu, Zhaohui S. Qin, and Xiao Sun. Integrative characterization of g-quadruplexes in the three-dimensional chromatin structure. *Epigenetics*, 14(9):894–911, 2019. PMID: 31177910.

[14] Julian L. Huppert and Shankar Balasubramanian. Prevalence of quadruplexes in the human genome. *Nucleic Acids Research*, 33(9):2908–2916, January 2005.

[15] Julian L. Huppert and Shankar Balasubramanian. G-quadruplexes in promoters throughout the human genome. *Nucleic Acids Research*, 35(2):406–413, December 2006.

[16] Eva Klimentova, Jakub Polacek, Petr Simecek, and Panagiotis Alexiou. PENGUINN: Precise exploration of nuclear G-quadruplexes using interpretable neural networks. *bioRxiv*, 2020.

[17] Fedor Kouzine, Damian Wojtowicz, Laura Baranello, Arito Yamane, Steevenson Nelson, Wolfgang Resch, Kyong-Rim Kieffer-Kwon, Craig J. Benham, Rafael Casellas, Teresa M. Przytycka, and David Levens. Permanganate/S1 nuclease footprinting reveals non-B DNA structures with regulatory potential across a mammalian genome. *Cell Systems*, 4(3):344–356.e7, Mar 2017.

[18] Shi-Qing Mao, Avazeh T. Ghanbarian, Jochen Spiegel, Sergio Martínez Cuesta, Dario Beraldi, Marco Di Antonio, Giovanni Marsico, Robert Hänsel-Hertsch, David Tannahill, and Shankar Balasubramanian. DNA G-quadruplex structures mold the DNA methylome. *Nature Structural & Molecular Biology*, 25(10):951–957, Oct 2018.

[19] Aline Marnef, Sarah Cohen, and Galle Legube. Transcription-coupled DNA double-strand break repair: Active genes need special care. *Journal of Molecular Biology*, 429(9):1277 – 1288, 2017.

[20] Anthony Mathelier, Oriol Fornes, David J. Arenillas, Chih-yu Chen, Grgoire Denay, Jessica Lee, Wenqiang Shi, Casper Shyr, Ge Tan, Rebecca Worsley-Hunt, Allen W. Zhang, François Parcy, Boris Lenhard, Albin Sandelin, and Wyeth W. Wasserman. JASPAR 2016: a major expansion and update of the open-access database of transcription factor binding profiles. *Nucleic Acids Research*, 44(D1):D110–D115, January 2016.

[21] Eun-Ang Raiber, Ramon Kranaster, Enid Lam, Mehran Nikan, and Shankar Balasubramanian. A non-canonical DNA structure is a binding motif for the transcription factor SP1 in vitro. *Nucleic Acids Research*, 40(4):1499–1508, 10 2011.

[22] Aleksandr B. Sahakyan, Vicki S. Chambers, Giovanni Marsico, Tobias Santner, Marco Di Antonio, and Shankar Balasubramanian. Machine learning model for sequence-driven DNA G-quadruplex formation. *Scientific Reports*, 7(1):14535, 2017.

[23] Dipankar Sen and Walter Gilbert. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. *Nature*, 334(6180):364–366, 1988.

[24] Jochen Spiegel, Santosh Adhikari, and Shankar Balasubramanian. The structure and function of DNA G-quadruplexes. *Trends in Chemistry*, January 2019.

[25] Quan Wang, Jia-quan Liu, Zhao Chen, Ke-wei Zheng, Chang-yue Chen, Yu-hua Hao, and Zheng Tan. G-quadruplex formation at the 3 end of telomere DNA inhibits its extension by telomerase, polymerase and unwinding by helicase. *Nucleic Acids Research*, 39(14):6229–6237, March 2011.

[26] James D. Watson and Francis H. Crick. A structure for deoxyribose nucleic acid. *Nature*, 171:737–738, April 1953.