Making of fusion genes in cancer: An in-silico study of mechanism of chromosomal translocations

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Chromosomal translocations involve exchange of genetic material between non-homologous chromosomes leading to the formation of a fusion gene with altered function. The clinical consequences of non-random and recurrent chromosomal translocations have been so well understood in carcinogenesis that they serve as diagnostic and prognostic markers and also help in therapy decisions, mainly in leukemia and lymphoma. However, the molecular mechanisms underlying these recurrent genetic exchanges are yet to be understood. Various approaches employed include the extent of the vicinity of the partner chromosomes in the nucleus, DNA sequences at the breakpoints, etc. The present study addresses the stability of DNA sequences at the breakpoint regions using in-silico approach in terms of physicochemical properties such as; AT%, flexibility, melting temperature, enthalpy, entropy, stacking energy and free energy. Changes in these properties may lead to instability of DNA which could affect gene expression in particular and genome organization in general. Our study indicates that the fusion sequences are comparatively more unstable and hence, more prone to breakage. Current study along with others could lead to developing a model for predicting breakage prone genomic regions using this novel in-silico approach.

Making of Fusion Genes in Cancer: An in silico study of mechanism of chromosomal translocations

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Abstract

Chromosomal translocations involve exchange of genetic material between non- homologous chromosomes leading to the formation of a fusion gene with altered function. The clinical consequences of non-random and recurrent chromosomal translocations have been so well understood in carcinogenesis that they serve as diagnostic and prognostic markers and also help in therapy decisions, mainly in leukemia and lymphoma. However, the molecular mechanisms underlying these recurrent genetic exchanges are yet to be understood. Various approaches employed include the extent of the vicinity of the partner chromosomes in the nucleus, DNA sequences at the breakpoints, etc. The present study addresses the stability of DNA sequences at the breakpoint regions using in-silico approach in terms of physicochemical properties such as; AT%, flexibility, melting temperature, enthalpy, entropy, stacking energy and free energy. Changes in these properties may lead to instability of DNA which could affect gene expression in particular and genome organization in general. Our study indicates that the fusion sequences are comparatively more unstable and hence, more prone to breakage. Current study along with others could lead to developing a model for predicting breakage prone genomic regions using this novel in-silico approach.

Keywords: Chromosomal translocations, carcinogenesis, instability of DNA, in-silico approach, physicochemical properties, fusion sequences, gene expression, prone to breakage

Introduction

Chromosomal translocations involve partial exchange of genetic material among non-homologous chromosomes[1]. The resulting chimeric fusion transcripts are reported to code for proteins that impart selective growth advantage thus triggering carcinogenesis[2]. The fusion transcripts and proteins are also characteristic markers of diagnosis and therapy[3]. Various leukemia and lymphoma subtypes are based on the type of chromosome translocation as per the WHO-2008 classification which is more clinically relevant as compared to the FAB classification. Some of the examples of significant oncogenic chromosomal fusions are t(9;22), t(15;17), t(8;21) and t(16;16). These translocations result in the formation of unique chimeric proteins which activate a number of signal pathways leading to malignant transformation by interfering with the basic cellular process, such as control of cell proliferation and differentiation, adhesion and cell survival[4, 18].

The fusion t(9;22) occurs between coding sequence of BCR gene on chromosome 22 with that of ABL1 gene on chromosome 9, hence result in derivative chromosome viz. Philadelphia

chromosome. The BCR-ABL fusion protein is a well-defined marker in Chronic Myeloid Leukemia[5].

The molecular analysis of Acute Promyelocytic Leukemia (APL) breakpoint has shown the involvement of RARA gene on chromosome 17 with PML gene on chromosome 15. Two reciprocal fusion products are formed, namely, PML/RARA and RARA/PML. Out of these, PML/RARA is implicated in leukemogenesis and may interfere with the normal functions of PML and/or RARA. APL is FAB M3 subtype of AML[6, 7].

The t(8;21) translocation is one of the most frequent chromosome abnormalities in AML and is morphologically associated with the FAB-M2 subtype of AML[5, 7]. It results in an in-frame fusion of two genes, AML1 and ETO. Due to translocation, the AML1 gene (also called RUNX1) on chromosome 21 fuses with the ETO gene (also referred to as the RUNX1T1) on chromosome 8[8]. The t(16:16) is a rare chromosomal abnormality in AML, also classified as AML- M4Eo according to FAB classification[9]. It involves the fusion of the CBFB (Core-Binding Factor Beta Subunit) gene at 16q22 with the smooth muscle myosin heavy chain (MYH11) at 16p13. This results in a generation of the CBFB-MYH11 fusion protein.

The occurrence of recurrent translocations can be hypothetically explained in various ways. We have earlier reported the study of physical proximity of chromosomes undergoing translocation[10]. In the present study, we address the properties of the breakpoint region of the chromosomes taking part in translocation as a possible factor playing a role in recurrent breakage and reunion of two chromosomal sites. The chromosomal sites involved in translocation are likely to play a role in translocation owing to their physicochemical properties such as AT%, flexibility, melting temperature, enthalpy, entropy, stacking energy and free energy. This was assessed along with a reference sequence that is flanking the breakpoint region. The information regarding physicochemical properties of gene sequences taking part in translocation could provide useful insights on the precise investigations of the mechanism of leukemogenesis.

The molecular and stochastic mechanism underlying the chromosomal translocations is still speculative and requires further studies involving varied chromosomal regions. The chromosomal translocations in leukemia are ideal candidates for such studies owing to the non-random and well-characterized breakpoints of the gene pairs that provide precise sequences for investigation. The availability of various computational approaches has generated a number of in-silico studies[11]. In one such study, a unique computational multi-parametric bio-informatics methodology was employed for analysis of genomic sequences of breakpoint coordinates, which

provided some perspective of molecular mechanisms of DNA strand break during translocation leading to epithelial carcinogenesis. This study led to the conceptualization of an algorithmic program which may predict possible breakpoint in any given sequence of human genome[12]. Appertaining to this prior study, we attempt to analyze the sequence properties of breakpoint regions of chromosome pairs taking part in translocation in selected four cases of leukemia subtypes that are known to be recurrent and also well characterized in terms of breakpoints. Three of the translocation pairs are non-homologous chromosomes (9/22, 8/21, and 15/17); whereas one translocation involves two different regions on the same chromosome on two different arms (16p/16q). Unlike the first three cases, the fourth case is unique as in this case the physical proximity of fusion partners is ascertained being on the same chromosome.

In the present study, we carried out in-silico analysis of DNA sequences present at the chromosome translocation breakpoints.

Materials and methods

Data mining

The in-silico analysis involved the use of various computational tools so as to obtain the fusion gene sequences and to acquire the physicochemical properties of the sequences flanking them (Figure 1).

Retrieval of the fusion sequences of BCR-ABL, PML-RARA, AML-ETO, and CBFB- MYH11, which are involved in the formation of fusion products, was done using TICdb (www.unav.es/genetica/TICdb). The retrieved fusion sequences were entered in BLAT (BLAST-like Alignment Tool) query box (http://genome.ucsc.edu/). From the BLAT search results, the ones showing maximum identity with reference sequence were selected. Maximum identity is the extent of similarity that the fusion sequence shows with the reference sequence of the human genome considered in the BLAT tool.

The UCSC genome browser was further used to retrieve 500 base pairs upstream and downstream (a 1000 bp sequence) of the breakpoints of the fusion gene product for both the partner chromosomes. Similarly, 50 base pairs upstream and downstream (a 100 bp sequence) of each gene were retrieved. In order to compare the sequences involved in the formation of fusion genes, exonic sequences of 1000 as well as 100 bp length were similarly retrieved for BCR, ABL, PML, RARA, AML1, ETO, MYH11, and CBFB these were taken as ‘control’.

Physicochemical properties

The physicochemical properties of breakpoint region are assumed to be responsible for the easy breakage and reunion with the non-homologous partner sequence; hence we hypothesized that the genomic regions neighboring the breakpoint of fusion sequences would be different in terms of physicochemical properties and hence selected 50 bps downstream and upstream of breakpoints of fusion sequence as internal controls. Similarly, in order to increase the sample size, a similar exercise was also done using long stretches of genomic sequence i.e. 500 bp upstream and downstream of breakpoints of fusion sequence as internal controls.

For the evaluation of the physicochemical properties of the fusion sequences obtained for all eight genes, including their exonic sequences, DiProGB (http://diprogb.flileibniz.de/) was used. The properties selected for evaluation were AT%, flexibility, melting temperature, enthalpy, entropy, free energy and stacking energy.

Results and Discussion

The total number of fusion sequences obtained for all four translocation pairs varied. For BCR-ABL fusion sequences obtained was 16, out of which those fusion sequences showing maximum identity were 12. While the total number of sequences obtained for PML-RARA was 26, out of which those with maximum identity were 18. For AML1-ETO 17 out of 37 sequences showed maximum sequence identity. And for CBFB-MYH11 all the 7 sequences showed sequence maximum identity.

Further comparison of values of the different physicochemical properties of the sequences involved in translocation and the controls was carried out as shown in table 1 and 2. The values shown are an average of all the 12, 18, 17 and 7 fusion sequences for the eight genes as well as their controls. Our data as shown in the tables and supplementary material suggest that the breakpoints of fusion sequence and controls had different AT% and related parameters as compared when compared with the two different base-pair lengthed internal controls. The extent of the difference in AT % and hence the related parameters was comparable for both, the 100 and 1000 bps internal controls.

Several physicochemical parameters such as melting temperature, stacking energy, free energy

etc. are measured as these are known to stabilize the DNA[13]. An evaluation of these factors can provide an explanation of various properties and functional interactions of DNA. These diverse factors that stabilize DNA act in concert to protect it from random breakage. If these factors remain consistent, they could prevent the genome from damage, but it is known that DNA is a dynamic entity and the confounding factors do show variation leading to DNA instability. The instability of DNA could affect gene expression in particular and genome organization in general[13]. Evaluation of the comprehensive data regarding physicochemical features of the fusion gene partners, when compared to the normal gene exons, showed variations in all the parameters (table 3).

Our results demonstrate that AT% is in direct proportion to the flexibility. The flexibility of DNA is important for its biological functions such as gene expression, gene regulation etc. It is influenced by several factors like sequence arrangement, temperature etc. It is well known that DNA flexibility depends on its sequence, which directly affects its stability[14, 15]. Numerous previous studies have shown that different sequence arrangements can greatly influence the stability of DNA. The AT-rich sequences are more flexible than the GC rich sequences. This increase in the AT%, as well as the flexibility of the sequences involved in the formation of fusion products in comparison to the controls, could indicate an increase in instability.

Melting temperature indicates the temperature at which half of the double-stranded DNA gets converted to single-stranded DNA. Since A and T are linked with each other with double hydrogen bonds, the DNA having higher AT content will have a relatively lower melting temperature. Also, the temperature can directly determine the state of the structure of DNA and thus greatly influence its flexibility[14, 15]. Our study correlates this decrease in the melting temperatures with an increase in flexibility and AT content for the fusion sequences.

As the hydrogen bonding between A and T would not alone lead to DNA instability, the stacking energy of the DNA could be taken into consideration. The stacking energy of the AT bases is generally weaker than the GC base pairs. Due to this, the AT-rich regions have a lower melting temperature as compared to the GC rich regions. The stacking energy hence can determine the DNA stability and its disruption can contribute to decrease in melting temperature and also change the thermal energy of the molecule. In our study, there was a significant decline in the stacking energy of the fusion sequences when compared to the stacking energy of the control[16].

Thermodynamics studies suggest that processes like the formation of double helix will happen spontaneously only if they result in a net increase in entropy[17]. There are reports which indicate

that more negative the free energy is, more is the stability of the DNA. On the basis of the relationship between standard free energy change (ΔG), enthalpy (ΔH°) and entropy (ΔS°), given by the equation, ΔG =ΔH° - TΔS°, we assume that a net decrease in free energy may result because of a decrease in enthalpy and entropy individually. Our results support this assumption; however additional assessment on the thermodynamic properties of DNA in relation to its structure needs to be carried out.

Conclusion

The mechanism underlying chromosomal translocations remains under speculation and requires advanced investigations. Based on our in-silico studies, the physicochemical parameters that are known to affect DNA stability viz., AT%, flexibility, melting temperature, stacking energy, enthalpy, entropy and free energy used for comparison of the sequences involved in formation of fusion products with the controls manifested instability of the sequences flanking the breakpoint regions. The overall decrease in AT% and flexibility and a gross increase in remaining properties indicated that these sequences were found to be more prone to breakage. Extensive work carried out in the direction of this novel approach could lead to the development of an in-silico predictive model that aids in finding a particular genomic area or region that is prone to frequent breakage.

Study Highlights

What is the current knowledge?

- Recurrent chromosome translocations leading to fusion genes are known to be pathogenic in cancer
- The mechanism underlying non-random involvement of breakpoints in gene pairs is not well understood despite various approaches reported in terms of the vicinity, DNA packaging, etc.

What is new here?

- The physicochemical properties of breakpoints in gene pairs participating in fusion are studied for instability favoring rearrangement
- The in-silico approach to study this mechanism in case of hematological malignancies has not been reported earlier

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Figure 1(on next page)

Flowchart depicting data retrieval of 500 and 50 base pairs upstream and downstream to the breakpoint flanking region of fusion gene pairs

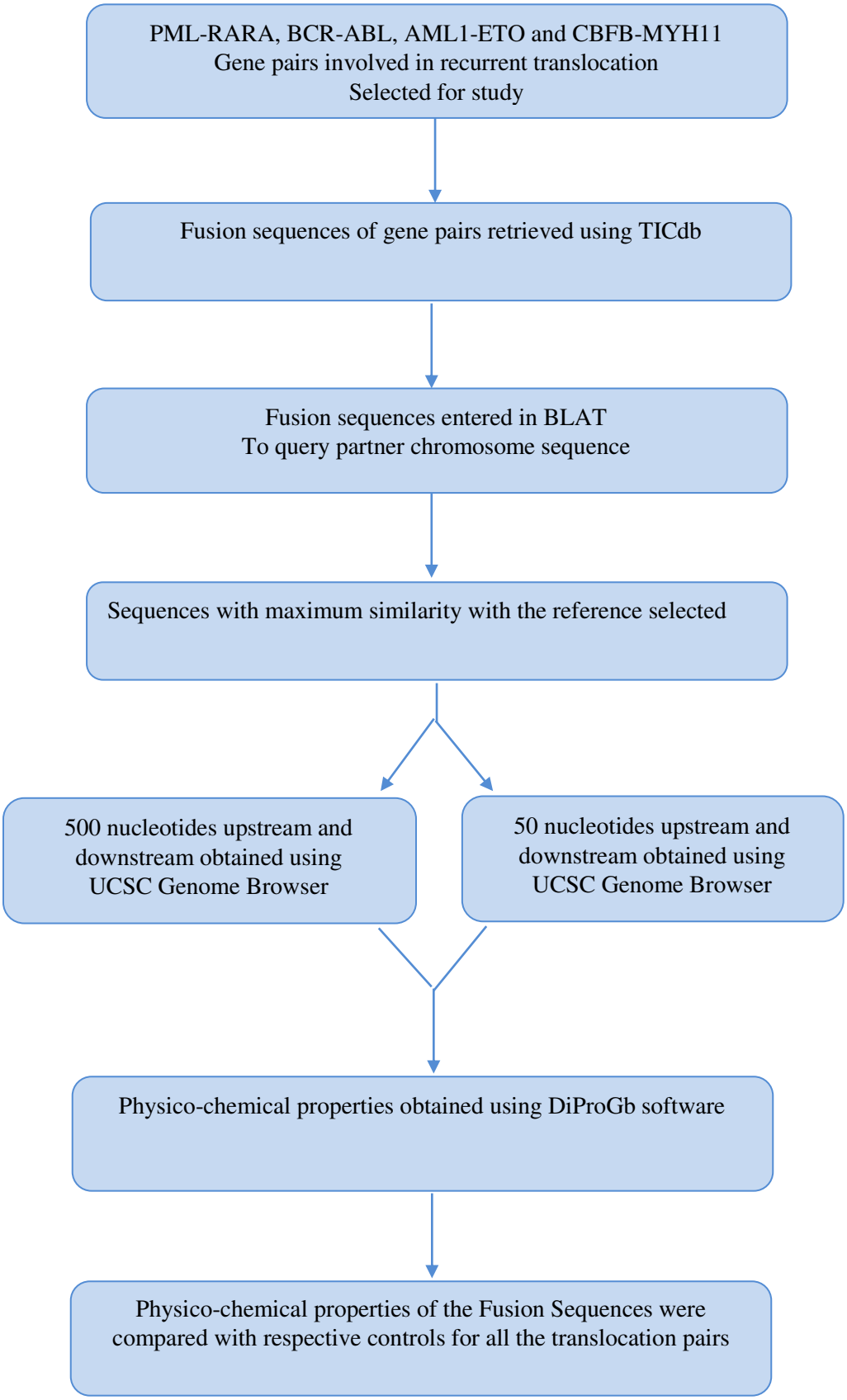

Figure 1: Flowchart depicting data retrieval of 500 and 50 base pairs upstream and downstream to the breakpoint flanking region of fusion gene pairs

Table 1(on next page)

Analysis of physico-chemical parameters/AT% within 1000 bps around breakpoint for Fusion Sequence (FS) and Control (C)

Average of all physico-chemical parameters/AT% within 1000 bps around breakpoint for Fusion Sequence (FS) and Control (C) for BCR, ABL, PML, RARA, AML1, ETO, CBFB, and MYH11

Table 1: Analysis of physico-chemical parameters/AT% within 1000 bps around breakpoint for Fusion Sequence (FS) and Control (C)

Gene	Sequence	AT (%)	Flexibility (kJ/mol A2)	Melting Temperature (°C)	Enthalpy (kcal/mol)	Entropy (cal/mol/K)	Stacking Energy (kcal/mol)	Free Energy (kcal/mol)
ABL	1000 FS	58.525	8.47	69.826	-8.318	-21.936	-7.567	-1.096
	1000 C	50.15	7.937	73.121	-8.558	-22.427	-7.947	-1.174
BCR	1000 FS	44.375	7.523	75.125	-8.7192	-22.779	-8.162	-1.223
	1000 C	44.2	7.47	75.451	-8.726	-22.773	-8.197	-1.231
PML	1000 FS	46.922	7.623	74.115	-8.593	-22.472	-8.05	-1.201
	1000 C	44.442	7.442	75.336	-8.687	-22.658	-8.195	-1.23
RARA	1000 FS	41.5	7.482	75.854	-8.808	-22.98	-8.166	-1.248
	1000 C	40.5142	7.265	76.785	-8.8485	-23.04	-8.32	-1.266
AML1	1000 FS	64.952	8.851	66.781	-8.06	-21.371	-7.25	-1.024
	1000 C	60.95	8.548	68.883	-8.211	-21.7	-7.5	-1.071
ETO	1000 FS	55.08	8.142	70.9	-8.358	-21.9	-7.707	-1.123
	1000 C	55.85	8.251	70.51	-8.353	-22.004	-7.66	-1.11
CBFB	1000 FS	64.95	8.85	66.781	-8.058	-21.371	-7.249	-1.024
	1000 C	60.96	8.548	68.88	-8.211	-21.7	-7.493	-1.071
MYH11	1000 FS	55.082	8.142	70.89	-8.3584	-21.99	-7.707	-1.1236
	1000 C	55.85	8.251	70.51	-8.353	-22.004	-7.657	-1.113

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Table 2(on next page)

Analysis of physico-chemical parameters/AT% within 100 bps around breakpoint for Fusion Sequence (FS) and Control (C)

Average of all physico-chemical parameters/AT% within 100 bps around breakpoint for Fusion Sequence (FS) and Control (C) for BCR, ABL, PML, RARA, AML1, ETO, CBFB and MYH11

Table 2: Analysis of physico-chemical parameters/AT% within 100 bps around breakpoint for Fusion Sequence (FS) and Control (C)

Gene	Sequence	AT(%)	Flexibility (kJ/molA2)	Melting Temperature (°C)	Enthalspy (kcal/mol)	Entropy (cal/mol/K)	Stacking Energy (kcal/mol)	Free Energy (kcal/mol)
ABL	100 FS	53.34	7.646	75.407	-8.768	-22.908	-8.202	-1.227
	100 C	44.1	7.397	75.582	-8.73	-22.78	-8.232	-1.234
BCR	100 FS	44.917	8.491	70.812	-8.378	-22.034	-7.676	-1.118
	100 C	43.2	7.57	75.63	-8.67	-22.6	-8.302	-1.227
PML	100 FS	45.56	7.478	75.155	-8.758	-22.88	-8.115	-1.229
	100 C	38.85	7.097	78.3	-8.97	-23.307	-8.56	-1.296
RARA	100 FS	43.78	7.682	74.912	-8.686	-22.683	-8.101	-1.225
	100 C	42.86	7.406	76.35	-8.79	-22.9	-8.316	-1.254
AML1	100 FS	66.94	8.983	65.833	-7.991	-21.234	-7.141	-1.003
	100 C	47.89	7.77	74.216	-8.632	-22.58	-8.091	-1.19
ETO	100 FS	55.059	8.0922	71.092	-8.348	-21.96	-7.76	-1.127
	100 C	43.25	7.28	76.28	-8.8	-22.937	-8.35	-1.246
CBFB	100 FS	52.857	8.207	72.095	-8.452	-22.205	-7.87	-1.146
	100 C	44.67	7.866	76.1	-8.81	-22.97	-8.362	-1.237
MYH11	100 FS	42.71	7.824	75.8	-8.771	-22.844	-8.247	-1.24
	100 C	37	7.38	77.29	-8.856	-22.97	-8.421	-1.269

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Table 3(on next page)

Overall comparison between Fusion sequences and Control

The table shows a general comparison of all the physico-chemical properties of all four fusion pairs with their respective exons for both 100bps as well as for 1000 bps. There is an overall increase in flexibility and AT content in the fusion sequences while other parameters like Stacking energy, free energy, entropy, enthalpy and melting temperatures show an overall decrease when compared to the control sequences.

Parameters	1000 bps		100 bps	
	Fusion sequence	Control	Fusion sequence	Control
AT (%)	↑	↓	↑	↓
Flexibility (kJ/molA$^{2)}$	↑	↓	↑	↓
Melting Temperature (°C)	↓	↑	↓	↑
Stacking Energy (kcal/mol)	↓	↑	↓	↑
Enthalpy (kcal/mol)	↓	↑	↓	↑
Entropy (cal/mol/K)	↓	↑	↓	↑
Free Energy (kcal/mol)	↓	↑	↓	↑

Table 3: Overall comparison between Fusion sequences and Control

↑ indicates an overall increase

↓ indicates an overall decrease