科研費 KAKENHI

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研究種目：若手研究（B）
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課題番号：２３７１０２３５
研究課題名（和文）　転写制御ネットワークのボトムアップ再構成のための新規実験／計算パイプラインの構築
研究課題名（英文）　Methods for the bottom-up reconstruction of gene-regulatory networks
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研究成果の概要（和文）：
・ChIP-chip と ChIP-seq ライブラリーから高効率にエンハンサー検索をする計算方法を開発(Fernandez and Miranda-Saavedra, 2012)
・転写制御モジュールの再構成のための新手法を開発(Hutchins et al, 2013)
・上記の手法により、抗炎症反応系を制御する STAT3 の基本機能を解明(Hutchins et al, Blood, 2012; Hutchins et al, JAK-STAT, 2013).

研究成果の概要（英文）：
• Development of the best computational method for enhancer detection, capable of identifying enhancers from ChIP-chip and ChIP-seq libraries with a better comparative performance than other published methods for enhancer prediction (Fernandez and Miranda-Saavedra, 2012).
• Development of a novel method for the reconstruction of Transcriptional Regulatory Modules (TRMs) (Hutchins et al, 2013).
• The above methods have provided new and fundamental insights into the STAT3 controlled anti-inflammatory response network (Hutchins et al, Blood, 2012; Hutchins et al, JAK-STAT, 2013).

交付決定額

（金額単位：円）

	直接経費	間接経費	合　計
交付決定額	3,500,000	1,050,000	4,550,000

研究分野：複合新領域
科研費の分科・細目：ゲノム科学・システムゲノム科学
キーワード：Enhancer identification, Transcriptional regulatory network, Epigenetics, Computational genomics, Genetic algorithm, Support vector machine, Transcriptional regulatory module.

１．研究開始当初の背景

(1) No general method exists for the bottom-up reconstruction of transcriptional regulatory networks, i.e. without knowing the most relevant transcription factors (TFs) involved.

(2) We need to understand not only where a key TF may bind but also understand what other transcription factors and co-factors assemble with the master TF to achieve genomic binding specificity.

２．研究の目的

(1) Develop methods that will facilitate the identification of the most important genomic and proteomic elements that will allow one to reconstruct transcriptional regulatory networks from the bottom-up.

３．研究の方法

(1) Computational analysis of next-generation sequencing data, protein-protein interaction data and bioinformatic predictions of TF binding sites.
(2) Artificial intelligence techniques (support vector machines, genetic algorithms).
(3) Experimental: Next-generation sequencing (ChIP-seq and RNA-seq) and standard biochemical and molecular biology techniques.

４．研究成果

The research plan for FY2011 included: (a) Location of the enhancer controlling the expression of the Fas receptor gene (Fas) upon T-cell receptor activation; (b) Experimental validation of enhancer function using enhancer-GFP stable transfects; (c) downstream computational analysis to identify putative regulatory transcription factors.

We tried to locate the enhancer regulating the expression of Fas by doing ChIP-seq for the epigenetic marks H3K4me1 and H3K4me3, which are characteristic of enhancers and transcription start sites (TSS), respectively. We also profiled p300 (a transcriptional co-activator and bona fide mark for enhancers) by ChIP-seq. The p300 experiment was not successful in our hands. However, the H3K4me1 and H3K4me3 ChIP-seq experiments were successful and allowed us to visually identify a number of peaks that were potential enhancers. Two such putative enhancers were cloned and their activity was determined using a luciferase assay. However, the result was ambiguous and we could not determine activity at these loci. We did further bioinformatics analysis of the ChIP-seq libraries that we generated and concluded that, although H3K4me1 and H3K4me3 are typically used in combination to discriminate enhancers from promoters, the identification of enhancers is in practice a much more complex task. We considered that the current experimental and computational methods for enhancer prediction from ChIP-chip/ChIP-seq data are not ideal because they either (i) consider a smaller number of marks than those necessary to define the various enhancer classes or (ii) work with an excessive number of marks, which is experimentally unviable. As part of this project and in order to overcome the limitations of the current methods for enhancer detection, we developed a new computational method for predicting enhancers from ChIP-chip or ChIP-seq

libraries called ChromaGenSVM (see publications).

We also produced a transcriptional profile of naïve and activated CD4+ T cells using RNA-seq (see publications).

In FY2012 We have continued working on the experimental identification of the enhancer(s) controlling the regulation of Fas, which has proven more difficult than previously thought. One important problem that we have anticipated is the identification of the transcription factors(s) (TFs) controlling the expression of Fas. TFs are known to combine with co-factors to form transcriptional regulatory modules (TRM), which are responsible for controlling gene expression programs with spatiotemporal specificity. To this end, we have developed a novel and generic method for the reconstruction of TRMs. Our method, rTRM, combines experimentally determined genomic sites with local motif enrichment analysis, cell type-specific expression data and protein-protein interactions. The selective advantage of rTRM over other methods lies in the incorporation of protein-protein interaction data as proteins need to physically interact with other proteins to perform their functions. Upon evaluation rTRM has been shown to be highly specific and allowed us to predict and experimentally verify specific and important interactions between E2F1 and STAT3 during the interleukin 10-mediated anti-inflammatory response in macrophages. Therefore rTRM is a novel and powerful method that will help us analyse the TRMs involved in controlling the expression of Fas and other genes in the near future (Diez, Hutchins and Miranda-Saavedra (2013) In review). In summary, in these two years of research we have significantly met the broad objective of the proposed project (“Methods for the bottom-up reconstruction of gene-regulatory networks”) and advanced the field of transcriptional regulation by developing novel and powerful computational tools, together with the experimental verification of a select set of important predictions. The reviews I have been invited to write by prestigious international journals (Briefings in Functional Genomics and JAK-STAT) are testimony to the international standing of our research.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計５件）

①Hutchins AP, Diez D, Miranda-Saavedra D.* Genomic and computational approaches to dissect the mechanisms of STAT3’s universal and cell type-specific functions. JAK-STAT. Peer-reviewed. 2013 (in press)

②Hutchins AP, Diez D, Takahashi Y, Ahmad S, Jauch R, Tremblay ML, Miranda-Saavedra D.* Distinct transcriptional regulatory modules underlie STAT3's cell type-independent and cell type-specific functions. Nucleic Acids Research.

Peer-Reviewed. 2013 Feb 1;41(4):2155-70. doi: 10.1093/nar/gks1300.

③ Hutchins AP, Poulain S, Fujii H, Miranda-Saavedra D.＊ Discovery and characterization of new transcripts from RNA-seq data in mouse CD4(+) T cells. Genomics. Peer-Reviewed. 2012 Nov;100(5):303-13. doi: 10.1016/j.ygeno.2012.07.014.

④ Fernández M, Miranda-Saavedra D.＊ Genome-wide enhancer prediction from epigenetic signatures using genetic algorithm-optimized support vector machines. Nucleic Acids Research. Peer-Reviewed. 2012 May;40(10):e77. doi: 10.1093/nar/gks149.

⑤Hutchins AP, Poulain S, Miranda-Saavedra D.＊ Genome-wide analysis of STAT3 binding in vivo predicts effectors of the anti-inflammatory response in macrophages. Blood. Peer-reviewed. 2012 Mar 29;119(13):e110-9. doi:10.1182/blood-2011-09-381483.

〔学会発表〕(計３件)

①Diego Miranda-Saavedra. Control of the anti-inflammatory response by STAT3. European Congress of Immunology 2012 (Glasgow). 6/9/2012.UK.

②Diego Miranda-Saavedra. Prediction of the effectors of the anti-inflammatory response by ChIP-seq and RNA-seq analysis. Information Processing in Cells and Tissues (IPCAT) Conference 2012. 1/4/2012. Trinity College, Cambridge.UK. (invited lecture by the conference board)

③ Diego Miranda-Saavedra. Genome-wide Enhancer Predictions from Chromatin Methylation Marks using Genetic Algorithm-Optimised Support Vector Machines. 2nd Next Generation Sequencing Asia Congress. 4 October 2011. Singapore.

６．研究組織

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