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論文内容の要旨

Biological robustness refers to the invariance of phenotypes in the face of perturbations including environmental fluctuations, mutations and stochastic noises in molecular interactions. There are different mechanisms underlying biological robustness. For instance, heat shock proteins, as molecular chaperone, prevent protein miss-folding at stress temperature; certain network architectures reduce the level of expression noise; and multiple signaling pathways often act in parallel and ensure normal development. However, we still know little about the full spectrum of mechanisms for biological robustness and how living systems have acquired them. To address these issues, here I focused on two mechanisms that confer robustness against perturbations: gene duplication and a repair mechanism for fate-map shift caused by extra copies of *bicoid* (*bcd*) gene in *Drosophila melanogaster*.

Gene duplication contributes to biological robustness by masking the effect of deleterious loss-of-function mutations. While previous theoretical studies of gene duplication have mainly focused on the gene silencing process after fixation, the process leading to fixation is more important for a newly arisen duplicated gene, because the majority of duplications would be lost before reaching a significant frequency in a population. In addition, it is generally accepted that non-heritable perturbations such as environmental fluctuations and stochastic noises are more important driving forces for evolution of robustness than heritable ones. In CHAPTER 2, I addressed whether a newly arisen single gene duplication can fix and be functionally preserved in a population under mutation pressure alone. From an analytical study and series of simulations, it was shown that thefixation probability with preservation of functional copies becomes twice the loss-of-function mutation rate (u_c) when the population size (N), the degree of dominance of mutations (h) and the recombination rate between the duplicate genes (c) are all sufficiently large ($Nu_c > 1$, $h > 0.1$, and $c > u_c$). This preservation of functional copies at both duplicated loci was observed for a long time. By contrast, when the gene is haplo-sufficient, one copy of the duplicates would lose its function soon after its origination. These results suggest that a large population tends to lose haplo-insufficient genes from the genome in the course of evolution. Acquisition of gene duplications throughout a genome will increase the chance for evolutional novelty.

While functional redundancy by gene duplication contributes to biological robustness, organisms have also acquired the ability to actively respond to heritable and non-heritable perturbations. Such an example can be seen in the repair of fate-map shift caused by extra copies of *bcd* genes in *Drosophila melanogaster* (CHAPTER 3). The maternal effect gene *bcd* serves as a morphogen and establishes the body pattern along the anterior-posterior axis in early embryo. Embryos from females carrying six copies of *bcd* genes (6x*bcd* condition) show the fate-map shift and

expansion of prospective head domain. Nevertheless, they still develop into almost normal adults, suggesting a repair mechanism for this fate-map shift. A previous study reported that cell death plays an important role in the repair of the expanded head region. However, there are many questions left unanswered. In this thesis, I addressed the following three issues. (i) Is there genetic variation in sensitivity to the 6x*bcd* condition among individuals? If so, then (ii) how much genetic variation exists? (iii) What is the genetic basis of excessive cell death occurring in this repair process?

In part 1 of CHAPTER 3, to address the first and the second issues, I established 40 second-chromosome strains derived from a natural population. The average relative viability of homozygous flies was 1.05 in the control condition of 2x*bcd* and 0.82 in the 6x*bcd* condition; the variance was 0.05 in 2x*bcd* and 0.10 6x*bcd*. Although both the average and variance showed significant differences between the two conditions, the wild-derived strains were generally resistant to the 6x*bcd* condition, indicating the importance of the repair mechanism in natural condition. In this variation survey, I obtained one strain that is highly resistant to the 6x*bcd* condition (r#109) and three strains that are sensitive to the fate-map shift (s#114, s#154 and s#254). These exceptional strains suggest that there is repair gene(s) that is required for normal development especially in the 6x*bcd* condition.

In part 2 of CHAPTER 3, I addressed the last question: the genetic basis of cell death in this repair process. To identify genes required in the fate-map shift repair, I firstly conducted two screenings: genetic screening by a series of deletion strains and microarray expression analysis. From the screening of 151 deficiency strains, I obtained two candidate genomic regions showing halo-insufficiency in the 6x*bcd* condition. From the microarray expression analysis, I found 83 genes up-regulated in the 6x*bcd* condition compared with the 2x*bcd* condition. Among these 83 up-regulated genes, 11 genes were up-regulated more than 2-fold and, indeed, one of them (*cg15479*) is located in one of the candidate genomic regions identified from deletion screening. Next, I tested the necessity of *cg15479* gene in the repair. This gene was expressed in the prospective head region in embryo and enhanced expression of *cg15479* was required for normal egg hatchability in the 6x*bcd* condition. I also conducted transgenic approach via GAL4-UAS system to reveal the function of *cg15479*. Ectopic expression of *cg15479* in imaginal discs reduced the size of wing and eye in a cell-autonomous manner and this size reduction was caused by caspase-independent cell death. Interestingly, while ectopic expression of *p53* can lead to death of any types of cell, induction of cell death by *cg15479* seems to depend on whether the cell fate is determined or not; cell death effectively occurred in proliferating or less differentiated tissues but not after the fate determination. Lastly, I found that the number of substitutions per site is lower for non-synonymous than for synonymous substitutions, indicating functional constraint on this gene. Taken together, the

present results suggest that *cg15479* plays a crucial role in active elimination of undesirable cells in this repair system.

For robustness, different mechanisms act at different levels; functional redundancy by duplicated genes acts at transcriptional level, while the repair mechanism for the fate-map shift acts at tissue or organ level. Multiple mechanisms act together to confer robustness and allow the accumulation of hidden genetic variation with a wide spectrum of mutations in natural populations. Exploring the hierarchical structure of biological robustness will be an important task for future research.

論文の審査結果の要旨

生物は外界の変動や遺伝子活性の変化が起こっても形質を一定に保つための緩衝機構を有している．田中君はRobustness（頑強性）と呼ばれるこの現象に興味を持ち，「進化の過程で緩衝機構が出現するにはどのような条件が必要か？」と「緩衝はどのような分子機構で担われているか？」という２つの観点から，進化遺伝学と発生遺伝学を統合した研究を行った．

まず，緩衝機構の一つである「同じ機能を持つ遺伝子が複数コピー存在する」という現象を取り上げ，ゲノムの一部分が重複した場合に重複した２つの遺伝子がたどる運命についてシミュレーションを行った．その結果，シミュレーションに「機能欠失型変異ヘテロ接合体の適応度」を取り入れれば，通常の遺伝子で推定されている優性の程度（h=0.02）や選択係数（s=0.1~1）の値を持つ遺伝子について重複遺伝子の両方のコピーの保持が可能であることを示した．従来のモデルでは同一機能の重複遺伝子は速やかに失われるとされていたのに対し，田中君の解析は，重複遺伝子が固定され，緩衝機構として働く可能性が充分にあることを初めて明らかにした重要な発見であり，すでに学術論文として発表している．

Robustnessの機構を解析するために，田中君は発生異常の「修復」に着目した．ショウジョウバエ胚の初期発生においては，bicoid（bcd）遺伝子のコピー数を増やすことによって胚の位置情報を変えて予定頭部領域が拡大した胚（6xbcd）を作っても，頭部領域で細胞死が起きることによって修復が行われ，正常な大きさの頭部を持つ個体に成長することが知られている．この修復現象に関与する分子機構を明らかにする目的で，田中君は遺伝的検索と分子的検索を組み合わせてゲノムレベルのスクリーニングを行い，塩基性ロイシンジッパー型転写因子をコードする新規遺伝子cg15479を同定した．6xbcd胚において，cg15479は予定頭部領域で過剰発現しており，cg15479の発現をRNA干渉法で抑制すると6xbcdの生存率が正常型に比べて有意に低下した．cg15479は既知の細胞死経路とは異なる経路で細胞死を誘導する活性を持つことも明らかにした．これらのことから，6xbcd胚では予定頭部領域でcg15479発現が増加し，細胞死を誘導することによって頭身バランスが修復されるとする分子機構を提案した．

cg15479の発現抑制は正常型の生存率をも低下させるので，cg15479の関与する細胞死経路は6xbcdでの修復以外でも利用されている可能性が高い．分子進化学的解析によって，田中君はcg15479が双翅目の共通祖先で出現し，その後純化淘汰を受け続けていることを明らかにした．遺伝子重複によるcg15479の出現の時期はハエの系統特有に生じたbicoidシステムの獲得の時期と近接していることから，bicoidシステムとcg15479のもたらすRobustness機能との間には重要な進化的関係性があることが示唆される．

田中君はRobustnessという最近着目されているテーマに新たな問題意識を持ち込み，発生生物学と集団遺伝学にまたがる領域でオリジナリティーの高い研究をなしとげた．

それぞれの分野で新しい成果を得ただけでなく，両分野を統合した解析や考察も行っている．さらに，研究の将来を展望して「Robustnessの種内多様性」という研究構想を築いており，すでにショウジョウバエの野外集団から修復力の高い系統，低い系統を得ている．生命科学の広い領域を俯瞰して研究テーマを設定する洞察力は特筆すべきである．以上の理由から，博士号授与の要件を満たすと審査員全員一致で判断した．