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学位論文題目　Genetic dissection of TFIIH function in Drosophila

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論文内容の要旨

Basal transcription factor TFIIH is a multi-functional protein complex that is involved not only in transcriptional initiation but also in DNA repair and cell cycle regulation. Among its nine subunits (Cdk7, CycH, Mat1, p62, p52, p44, p34, XPB, XPD) are those that have enzymatic functions such as kinase, ATPase and helicase activities (Cdk7, XPB, XPD), which are likely to be used differentially depending on the state of the cell. Cdk7 functions in the control of cell cycle as a trisubunit complex CAK (Cdk-activating kinase, Cdk7/CycH/Mat1), or in the phosphorylation of RNA polymerase CTD as the TFIIH holoenzyme (TFIIH kinase). On the other hand, XPB and XPD functions to unwind the double strand of DNA in transcriptional initiation (promoter opening or promoter clearance) or DNA repair. Furthermore, it has recently been known that TFIIH kinase also functions in E2F degradation, as well as in the phosphorylation and activation of nuclear receptors. Since most studies concerning TFIIH have so far been done by biochemical analyses, it is completely unknown how the TFIIH activity is regulated when three functions operate simultaneously.

For the multi-subunit molecules like TFIIH, it is thought that the conformation is important for its function. If the 'core' subunit (not an enzymatic subunit) of such a molecule is changed, what structural and functional change will be caused? In order to approach the regulatory mechanism of TFIIH function, I investigated the role of the p52 subunit, which is one of the core subunits of TFIIH, in developing *Drosophila*. It has previously been demonstrated that a deletion of the C-terminal region (60 a.a.) of p52 lacks NER (nucleotide excision repair) activity in yeast, and injection of anti-p52 antibody to human fibroblast induces the defect of NER and transcriptional activity. More recently, it has been shown that a recombinant TFIIH from which the C-terminal domain of p52 is deleted exhibits the defects in both DNA repair and transcriptional activities. To learn the function of p52 during development I identified the loss of function alleles of *Drosophila* p52 and found that a previously identified mutant, marionette (*mrn*), fail to complement the lethality of these alleles. All alleles of p52/*mrn* were larval lethal and died before reaching the mature third instar larva. p52/*mrn* larvae exhibited abnormal feeding behaviour suggesting a defect in neural development. To analyse p52/MRN function further, I made somatic clones in the developing eye for null and hypomorphic alleles using the mitotic recombination technique. The results suggested that p52/MRN is required for cell proliferation or viability.

Since the major function of TFIIH is considered to be in transcription, phenotypes observed in the clone of p52/*mrn* $^{-/-}$ might be based on the defect in transcription. First, I checked the transcription in the p52/*mrn* $^{-/-}$ mosaic eye using various cell cycle and cell fate markers. However, I could not detect any defects in transcription; this result differs from those obtained by *in vitro* experiments. It suggests that under my experimental condition TFIIH could exert its activity of transcriptional initiation in the absence of p52/MRN.

In addition to transcriptional initiation, TFIIH is also involved in DNA repair mechanism called NER. NER-deficient cells are generally hypersensitive to the UV light and its irradiation triggers apoptosis. I thus checked the UV sensitivity of p52/*mrn* cells. In contrast to wild type cells that hardly showed any signs of apoptosis upon UV irradiation, massive apoptosis was induced within the p52/*mrn*

$^{-/-}$ clones. It has been known that DNA damage induces G1 or S, G2 arrest depending on the cell cycle phase, when the damage is generated. Failure to repair naturally occurs DNA damage is unlikely the cause of the cell cycle defect, because mutants of NER components show almost normal development and DNA synthesis. The abnormality of cell proliferation is thus not a secondary consequence of the NER defect.

In order to know more detail about the function in cell proliferation, I checked cell cycle progression in p52/*mrn* $^{-/-}$ clones in the eye. In p52/*mrn* mutant clone, the progression of cell cycle was slow anterior to the morphogenetic furrow, a moving front of differentiation where G1 arrest takes place. A block of G1/S transition occurred just behind the morphogenetic furrow; in spite of the accumulation of CycE that can trigger the entry into the S-phase, the lack of BrdU incorporation indicated that G1/S switch was not activated.

Taken together, I showed that p52/MRN is important for the cell proliferation and viability in the developing tissue. Since p52/MRN is one of 'core' subunits of TFIIH, it is possible that its absence changes the conformation of the TFIIH complex and affects its enzymatic activity. To identify the enzymatic activity that depends on the presence of the p52/MRN, I compared the loss of function phenotypes of p52/*mrn* to those of *cdk7* and *xpb /hay*, which are two of the three-enzymatic subunits of TFIIH.

First, I checked the CAK activity in *cdk7* mutant in eye imaginal disc. In contrast to yeast CAK, which triggers G1/S and G2/M transition, the requirement of metazoan Cdk7 in G1/S transition has not been demonstrated. While my work using *cdk7* ts suggested that the required amount of CAK activities between the two transitions are differ, and that the G1/S transition requires less CAK activity than the G2/M transition. Furthermore, I checked the somatic clones of *xpb /hay* $^{-/-}$ in the developing eye and identified a failure of G1/S transition as p52/*mrn*. These similar phenotypes suggest that not only CAK trisubunit but also other subunits of the TFIIH complex are necessary for the G1/S transition.

How is TFIIH involved in the cell cycle? While CAK trisubunit has been shown to be sufficient to direct cell cycle progression in *in vitro* experiments, there are situations where Cdk7 exhibits kinase activity not as a CAK trisubunit but as a TFIIH kinase: E2F degradation, nuclear receptor activation, and phosphorylation of CTD. There are several physical interaction data between p52 and XPB. XPB interacts not only with p52 but also with Cdk7 and Mat1. Moreover, in my study, the loss of function mutants of *Drosophila xpb*, *haywire* showed cell cycle abnormality similar to that of p52/*mrn* and *cdk7*. These observations suggest a possibility that p52 affects Cdk7 activity *via* XPB. Probably the lack of p52/MRN might change the activity of XPB, or its stability in a complex, and affect the activity of CAK as a result.

This mechanism that p52 affects CAK activity through XPB, however, is unlikely to function constitutively. Although p52/*mrn* showed almost wide spread expression in the embryo and the imaginal discs during development, an elevated level of expression was seen in the regions of active cell proliferation. During eye development p52/*mrn* was highly expressed in G1 arrested cells in the morphogenetic furrow. So, it is thought that p52/*mrn* level might change during the cell cycle by receiving a differentiation signal, and increases the CAK activity when its expression rise. The

uncoupling of normal cell cycle and differentiation in p52/*mm* mutant clone suggests that TFIIH might be a key molecule that connects cell cycle and differentiation.

論文の審査結果の要旨

基本転写因子 TFIIH は protein kinase, helicase といった酵素活性を持つ３つのサブユニットを含む全体として９つのサブユニットから構成される。そして転写因子としての機能だけでなく、DNA 修復や細胞周期の制御といった様々な生理活性を担っている。これまでの TFIIH に関する研究は生化学的解析が中心であり、これら９つのサブユニットが生体内における各々の生理活性にどのように関わっているかは不明の点が多い。松野さんは TFIIH の生体内における機能を明らかにする目的でショウジョウバエを用いてコアサブユニットである p52 と helicase 活性をもつ XPB サブユニット、kinase 活性をもつ CDK7 サブユニットの遺伝解析を行った。これまで生化学的解析から、TFIIH の細胞周期制御機能には CDK7 を含む３つのサブユニットから成る CAK (CDK-activating kinase)が必要かつ充分であるとされてきたが、CAK に含まれない p52 や XPB の機能欠失型変異株においても同様の細胞周期の異常が観察された。この結果は、生体内における細胞周期制御には、CAK 単独でなく THIIH 複合体としての integrity が必要なことを示しており注目に値する。さらに松野さんは、p52 サブユニットが生体内で転写より、ヌクレオチド除去修復や細胞周期制御に重要な役割を果すことを明らかにした。
以上のように、この論文の内容は生体内における TFIIH 複合体の機能を多細胞生物の系で初めて明らかにしたもので、遺伝学専攻の博士論文としての条件を満たすことを審査員全員が認めた。

博士論文審査に関わる公開発表会の後に、論文審査員と松野さんの間で質疑応答がなされた。その結果、松野さんは博士論文に関わる研究分野および関連する研究分野について充分な知識をもっており、その知識にもとずいて考察する能力をそなえていることがわかった。また、本論文は英語で書かれており、学位にふさわしい英語の能力をもつと判断した。