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RESEARCH HIGHLIGHT

When inordinate tissue growth is beneficial: Improving silk production by increasing silk gland size

Xavier Bellés[1]

[1]Institute of Evolutionary Biology (CSIC-UPF), Passeig Marítim 37, 08003 Barcelona, Spain

Some 5 000 years ago, the legendary Chinese emperor Huang-Ti ordered his wife, Hsi-Ling-Shi, to find out what was damaging his mulberry trees. Hsi-Ling-Shi found white worms eating the mulberry leaves and spinning shiny cocoons. She accidentally dropped a cocoon into her tea cup and saw a delicate, slender thread unwind itself from the cocoon. The young princess had discovered silk [1, 2].

As with many agricultural crops and livestock, the silkworm (*Bombyx mori*) has acquired the ability to produce large amounts of silk proteins as a result of selective breeding over more than 4 000 years. Silk, with a production volume of some 70 000 tonnes per year [3], is mainly used in the textile industry and represents a large-scale business worldwide. The increasing demand has driven notable efforts to improve silk production through hybrid breeding, a strategy that has been very successful during the past century [3], but silk production has reached the long-expected plateau, which is consubstantial to the empirical breeding approach. However, recent work by Sheng Li and associates [4] has helped to overcome this sericultural bottleneck.

The silk gland of the silkworm is divided into three morphologically and functionally distinct regions, known as posterior, middle and anterior silk gland [5]. The main constituent of silk is a protein known as fibroin, which is found in the core of the silk thread and is synthesized by the posterior silk gland. This fibroin core is covered with a layer of protein called sericin, which is produced in the middle silk gland [3, 5]. The weight contents of fibroin and sericin in total silk proteins are approximately 75% and 25%, respectively. The strategy planned by Sheng Li and his team [4] was to generate transgenic silkworms with increased Ras activity in the posterior silk gland in order to obtain silkworms with this part of the gland enlarged and thus able to produce more fibroin. Indeed, this sort of biotechnological brute-force approach gave the desired results.

Ras oncogenes are well-known to be key players in tumorigenesis. Amongst other effects, the activation of Ras signaling causes cell growth, whereas dysregulation can ultimately lead to oncogenesis and cancer [6]. Indeed, expression of activated Ras specifically in the larval prothoracic gland in the fruit fly (*Drosophila melanogaster*) increased gland size and resulted in a precocious release of molting hormone [7]. In this contribution, Philip Caldwell and colleagues used the conventional *Drosophila* Gal4/UAS system to modulate Ras signaling specifically in the prothoracic gland cells [7]. In the case of *B. mori*, which had been reluctant to genetic transformation for long time, an equivalent system for germline transgenesis using a *piggyBack* transposon-derived vector was developed in 2000 [8]. It was successfully used, for example, by Takahiro Shiotsuki and associates to study the regulatory role of juvenile hormone (JH) esterase on the degradation of JH in *B. mori* [9]. The results were spectacular: overexpression of JH esterase from the embryo stage resulted in steady degradation of JH and, as a consequence, in larval-pupal metamorphosis after the third larval instar, two instars earlier than in wild-type silkworms [9].

Li's team [4] used the same *Bombyx* binary Gal4/UAS system to overexpress *Ras1CA* oncogene in the posterior silk gland, and found that size of this gland region increased by almost 60% in transgenic silkworms with respect to controls, whereas silk production also increased by approximately 60%. As expected, food consumption also augmented, but only by 20%, thus resulting in an estimated 30% improvement in the conversion efficiency of food to silk. Subsequently, researchers embarked on an intensive study of the mechanisms underlying the observed phenotype (Figure 1) [4]. They showed that *Ras1CA* overexpression in the posterior silk gland of transgenic silkworm increased Ras activity and stimulated the phosphorylation of Raf and PI3K110,

Correspondence: Xavier Bellés
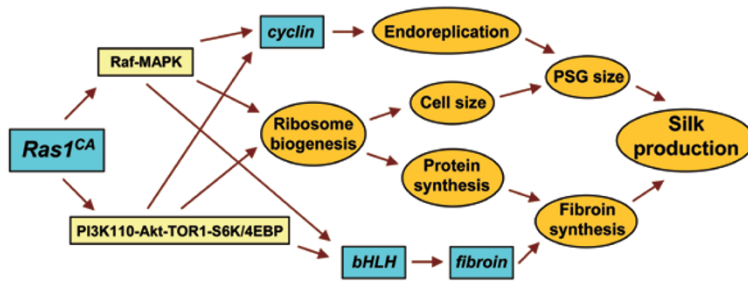E-mail: xavier.belles@ibe.upf-csic.es

Figure 1 Basic mechanisms underlying the effect of overexpressing the onco-gene *Ras1^CA* in the silworm posterior silk gland (PSK) on silk yield increase.

which in turn activated the Raf-MAPK and PI3K-Akt-TORC1-S6K/4EBP pathways. Subsequently, these pathways up-regulated the cyclins involved in endoreplication, thus promoting endoreplicative cycles that increased DNA content and contributed to the size increase of the posterior silk gland. In parallel, as a consequence of activation of the PI3K-Akt-TORC1-S6K/4EBP pathways, ribosome biogenesis and protein synthesis were enhanced. Finally, Ras activation up-regulated bHLH, a transcription factor that is specifically expressed in the silk gland and enhances fibroin gene expression, resulting in a higher fibroin production and silk yield (Figure 1) [4].

In summary, a case of tissue hypertrophy induction that produced beneficial results. Indeed, as the posterior silk gland, where the overexpression of *Ras1^CA* oncogene was driven, specializes in the production of fibroin, the overproduction of this protein can explain the resulting increase in silk production. A question then arises concerning the quality of the silk obtained, especially as the relative proportion of sericin, which is produced by the middle silk gland and forms the external silk layer, decreases. This potential silk-quality problem, however, could also be solved using a biotechnological approach. Moreover, the *B. mori* specimens used in the experiments were from the laboratory strain Dazao, which is useful as a model for experimental work but is not very productive. Thus, transgenic breeding should be carried out using a hybrid silkworm strain, as usually occurs in sericulture, for silk production on a commercial scale. Therefore, there are still various issues to be resolved before it can be proclaimed that the transgenic silkworms obtained by Li and associates are the panacea, but it is clear that they have great potential for use in sericulture and may pave the way for further improvement and refining. Indeed, obtaining cocoons 60% bigger than normal is no trifle. King-size cocoons that would not have fit into the delicate tea cup of the princess Hsi-Ling-Shi.

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