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Caenorhabditis elegans as a chemical screening tool for the study of neuromuscular disorders. Manual and semi-automated methods

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ABSTRACT

We previously reported the use of the cheap and fast-growing nematode *Caenorhabditis elegans* to search for molecules, which reduce muscle degeneration in a model for Duchenne Muscular Dystrophy (DMD). We showed that Prednisone, a steroid that is generally prescribed as a palliative treatment to DMD patients, also reduced muscle degeneration in the *C. elegans* DMD model. We further showed that this strategy could lead to the discovery of new and unsuspected small molecules, which have been further validated in a mammalian model of DMD, i.e. the *mdx* mouse model. These proof-of-principles demonstrate that *C. elegans* can serve as a screening tool to search for drugs against neuromuscular disorders. Here, we report and discuss two methodologies used to screen chemical libraries for drugs against muscle disorders in *C. elegans*. We first describe a manual method used to find drugs against DMD. We further present a semi-automated method, which is currently in use for the search of drugs against the Schwartz–Jampel Syndrome (SJS). Both assays are simple to implement and can be readily transposed and/or adapted to screens against other muscle/neuromuscular diseases, which can be modeled in the worm.

Finally we discuss, with respect to our experience and knowledge, the different parameters that have to be taken into account before choosing one or the other method.

1. Introduction

Duchenne Muscular Dystrophy (DMD) or Schwartz–Jampel Syndrome (SJS) are rare inherited neuromuscular disorders. Despite the identification of the genes responsible of these diseases [1,2], their physiopathology is still poorly understood, thus hindering the development of pharmacological therapies.

The identification of chemical molecules beneficial to patients suffering from rare inherited diseases requires efficient screening strategies. The setups of traditional pharmacological *in vitro* screening systems are usually based on the binding or the action of drugs on specific target proteins [3]. Since for most rare diseases the mechanisms that lead to their establishment are unknown, it is difficult to target relevant proteins or pathways. Moreover, muscle diseases usually need the complexity of a whole organism and movement to be initiated; therefore the development of relevant high content cell culture screening systems is mostly impossible. Finally, murine or other mammalian models, which are now available for most inherited muscle diseases [4,5], are not well suited to large-scale experiments like chemical screening because of their long breeding time and high costs.

A promising alternative to traditional *in vitro* and cellular systems is to use small model organisms like the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* or the zebrafish *Danio rerio*, which allow medium to high throughput screening of thousands of molecules at a low cost [6]. Such models can be used as first pass filters to identify molecules that can be further tested in mammalian models.

C. elegans, in particular, has many advantages with respect to the investigation of inherited neuromuscular diseases. More than 50% of human genes have counterparts in the *C. elegans* genome, among them many genes responsible for human genetic diseases [7]. In addition to this high conservation of genes, signaling pathways are in general well conserved and some of the *C. elegans* organs, most notably muscles, have a cellular physiology similar to that of vertebrates. *C. elegans* has striated and non-striated muscles. Non-striated muscles include pharyngeal, intestinal, uterine, vulval and anal muscles, while the body wall muscles are striated (Fig. 1A). Body-wall muscles are required for the movement of the worm; they are distributed in four longitudinal bands, named quadrants that run from head to tail. Each quadrant is formed by a single layer of diamond shaped muscle cells. The overall structure, composition and physiology of these striated muscle cells

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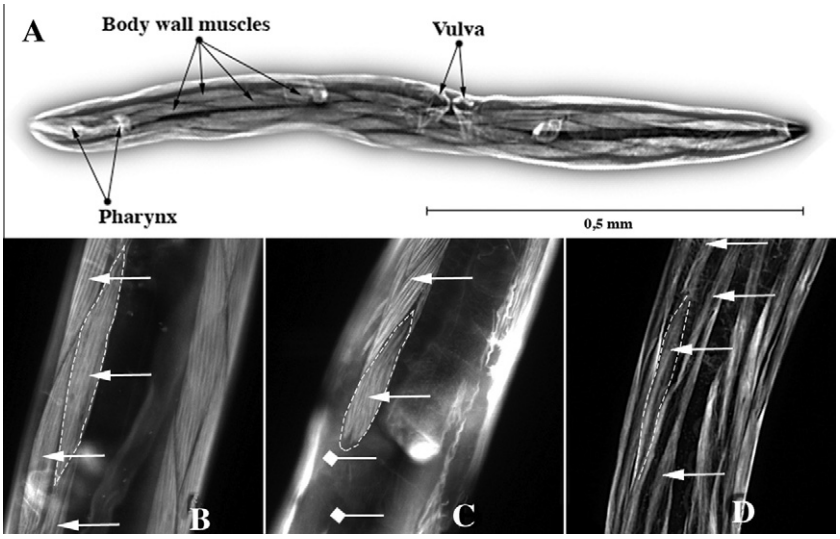

Fig. 1. Muscle cells in *C. elegans*. Images of *C. elegans* muscle cells after a phalloidin–rhodamine staining. A: Whole animal image. *C. elegans* has striated and non-striated muscles. Pharyngeal, vulval and anal muscle cells (not shown) are non-striated, while the body-wall muscle cells are striated. Body-wall muscle cells are distributed in four longitudinal bands, called quadrants that run from head to tail. Each quadrant is formed by a single layer of diamond shaped mononucleated cells. B–D: Body-wall muscle cells in wild type and *C. elegans* mutants. Muscle cells are indicated by arrows and delimited by a disrupted line. Absent cells are indicated by diamond arrows (C). In comparison to wild type muscle cells (B), muscle cells from the SJS model (D) are thinner but do not disappear as in the DMD model (C).

are close to vertebrate skeletal muscles and especially sarcomeric components are well conserved during evolution [8]. The major differences of *C. elegans* striated muscles with respect to vertebrate striated muscles are that muscle cells do not fuse and remain mono-nucleated and that *C. elegans* lacks regenerative processes.

Finally, the small size, the short life cycle and the simple and low-cost growth conditions of *Caenorhabditis elegans* allow for large scale studies such as chemical screening [6]. Indeed, *C. elegans* can be grown in multi-well plates and specific automated pipetting systems can be used at all developmental stages [6,9]. Moreover, combined with fluorescent markers, the optical transparency of the worm allows for the detection of functional and morphological abnormalities or changes in living worms. Several systems already exist to record *in vivo* fluorescence at a cellular or sub-cellular level [6].

Here, we present and discuss two screening methods used to search for small molecules against muscle disorders in *C. elegans*. First, we describe a method we used to find beneficial drugs against muscle degeneration in a *C. elegans* DMD model. This method is fully manual but really easy and cheap to set up and to perform. Secondly, we present a semi-automated experiment, which is currently in use for the screen of drugs beneficial to a *C. elegans* model of SJS. Both methods are simple to implement and can be readily transposed and/or adapted to screens for molecules on other muscular/neuromuscular diseases modeled in *C. elegans*. Depending on the phenotype to observe and the available equipment, a wide variety of readouts can be easily integrated into these procedures, such as automated imaging and automated locomotion measurements [6]. Finally we discuss the advantages and limits of each of these methods with respect to our experience.

1.1. General screening strategy

The screening strategy for the screens we have performed includes the following steps:

- Development of a pertinent *C. elegans* model,
- Set up of culture conditions and readouts sufficiently robust and in accordance with a large-scale screening campaign (time, workload),
- Screening in duplicates,
- Optional: secondary screening to confirm the first pass hits,
- Validation of hits.

The hit validation step consists in confirming the results of the screening step by reproducing the experiment with a different readout, usually a more direct and more detailed observation.

1.2. Diseases and models background information

1.2.1. Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy is a muscle wasting disease caused by the absence of dystrophin. Its physiopathology is still a matter of debate. Currently, the only pharmacological treatment proposed to DMD patients is Prednisone, a steroid, which slightly slows down muscle degeneration [10].

In order to develop more efficient pharmacological treatments, chemical screens on an appropriate model are needed. Different mammalian models of DMD exist, most notably the *mdx* mouse and the GRMD dog [11,12]. However, as mentioned above, mammalian models are not suitable for large-scale experiments like chemical screening. Muscle cell cultures are not suitable either because they do not recapitulate the muscle degeneration phenotype of DMD.

Several years ago our group identified a mutation in the *C. elegans* homolog of the dystrophin gene: *dys-1*(*cx18*), which leads to a phenotype of hyperactivity and slight muscle degeneration [13,14]. Muscle degeneration could be increased by combining the *dys-1*(*cx18*) mutation with a thermo-sensitive mutation in the *hlh-1* gene, the homolog of the myogenic factor MyoD. The *dys-1*(*cx18*); *hlh-1*(*cc561ts*) double mutants (strain LS587) become paralyzed in a time-and activity-dependant manner, due to progressive muscle degeneration resulting in muscle cells loss (Fig. 1C) [14]. It will be called here after *C. elegans* DMD model because it mimics the muscle wasting seen in DMD patients.

In a previous study, we reported that Prednisone, which is generally prescribed as palliative treatment to DMD patients, reduced muscle degeneration in *C. elegans* [15]. This was the first proof-of-principle that *C. elegans* can serve as a chemical screening tool to find candidate molecules against muscle disorders. We further showed that this strategy could lead to the discovery of unsuspected small molecules able to reduce muscle degeneration, which