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Review Article

Searching for Early-Onset Protein Aggregation to Identify Longevity Mutations

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Abstract

Genetic screening techniques are frequently employed to find the genes responsible for particular biological processes. Forward genetic screens in Caenorhabditis elegans rely on the discovery of mutants that are capable of self-propagating clonal colonies and are thus reproductively active (Zhou et al., 2014). Thus, it is difficult to develop screens that focus on post-reproductive characteristics, including longevity. We accomplish high-throughput automated screening for short-lived mutants utilising protein aggregation as a marker for ageing using microfluidic technology and image processing. Using fluorescently-labeled PAB-1 as a readout, we use microfluidics to undertake repetitive high-throughput analysis and sorting of animals with enhanced protein aggregation as well as to maintain a reproductively active adult mutagenized population (Hermann et al., 1999). We show that longevity mutants may be found without conditional sterilisation or manual separation of the parental and progeny populations by quantitatively analysing fluorescently labelled aggregates and screening for accelerated protein aggregation. We further demonstrate that the aggregate shape of elderly wildtypes and premature aggregation mutants differs; indicating that aggregate growth is time-dependent.

INTRODUCTION

A complex web of genetic and environmental variables controls the process through which organisms experience structural and functional degradation as they age. As a larger portion of the global population approaches old age, gaining a better knowledge of the ageing process becomes more important to human health every year. The identification of the genetic pathways at work is a crucial first step in comprehending the molecular processes that control ageing. By looking for genes that control lifespan, the worm C. elegans has been essential for identifying the genetic pathways that affect longevity. It is possible to examine the genotype-phenotype landscape and find uncharacterized genes that control ageing using methods for genetic screening that rely on novel phenotypes or the creation of new tools. Due to its short lifespan, genetic tractability, quick life cycle, and simplicity of culture, C. elegans is the best model system to do large-scale genetic screening of ageing regulators. Both forward and reverse genetic screens, which use random mutagenesis or targeted gene silencing, are applicable to C. elegans (Tot et al., 2006). By giving populations of C. elegans a library of dsRNA-expressing bacteria, large-scale reverse genetic RNA interference (RNAi) screenings may be performed. Important pathways, such those related to metabolism and mitochondrial function, have been discovered using RNAi screens for longevity genes, however this requires handling and lifelong monitoring of enormous populations. In order to avoid the necessity to segregate adults from their progeny, these screenings are often performed on animals that have undergone genetic modification or exposure to 5-fluorodexoyuridine (FUdR). While forward genetic screens rely on random mutagenesis and can produce potent and varied functional mutations, RNAi screens solely allow for the evaluation of the consequences of gene silencing. A temperature-sensitive mutant that becomes sterile at 25C was used in the first forward genetic screen for longevity mutants because it enables the observation of population survival without reproduction (Borgquist et al., 2015).

Recently, populations resulting from individually mutagenized animals were tested using conditionally sterile

animals in a forward genetic screen for maximal population lifetime. These methods require measuring the lifespan of each mutant's clonal population in order to study it, just as RNAi, and are therefore quite time-consuming (Shrout 1998).

Traditional forward genetic screens focus on isolating individuals from a population that has been mutagenized so that clonal populations may be created and the phenotypic can be examined. This method cannot be used to screen for mutations that affect longevity because putative mutants must be isolated while they are still capable of reproducing. As screening traits, alternative methods have included correlations or predictors of ageing (Barbarich et al., 2013). In one study, it was determined which animals had decreased motility at the very last phases of reproduction but normal locomotion rates at the start of adulthood. Recently, animals in the late stages of reproduction were screened for the presence of individuals with elevated levels of intestinal autofluorescence from lipofuscin, a substance thought to be a sign of ageing. By employing FUdR to stop embryonic development, the mutagenized population of animals was kept synchronised up to the late phases of reproduction. Worms resumed reproduction and permitted progeny collection once they were no longer exposed to FuDR. Screening animals that can naturally breed might result in the identification of ageing genetic pathways that have remained elusive up until now (Aoki et al., 2012). Reproduction and FUdR exposure are known to influence variations in lifespan under specific circumstances. Genetic screening in the late stages of reproduction has not previously been accomplished because it is labor-intensive to separate mutagenized parents from their offspring (Giordano et al., 2001).

Increased levels of protein aggregation as a result of a reduction in proteostasis is one trait seen in older people. Increased levels of aggregation in PolyQ illness models have been utilised in screens to look for genes that control proteostasis. Protein aggregation hasn't, however, been employed as an age-related indicator thus far. The Poly(A)-binding protein PAB-1 is one protein whose aggregation has been demonstrated to increase with ageing. In contrast to PolyQ, PAB-1 is spontaneously expressed and aggregates in wild-type C. elegans. Since PAB-1 is a stress-granule protein, aggregates are seen when stress levels are elevated. Additionally, it has been found that aggregation rises significantly with age, peaking just after the reproductive season (Connan et al., 2006). Thus, we postulated that mutants with a faster rate of ageing may be found by randomly mutating animals and looking for enhanced PAB-1 aggregation in the late phases of reproduction. The isolation of actual ageing mutants would be made possible by quantifying the lifetime of putative mutants, even though this method might also produce mutants with altered stress-granule production. Furthermore, in the absence of exogenous stress, mutants with altered sensitivity to stress, which might similarly be detected by assessing PAB-1 aggregation, would probably be harder to spot. In this study, we created a novel screening method that targets protein aggregation to identify transient C. elegans mutants with elevated levels of protein aggregation (Beadle et al., 2015). By combining microfluidic devices for progeny removal and animal sorting with quantitative image analysis and automated scoring of protein aggregation levels, we screened mutagenized animals in the late reproductive stage. We acquired a collection of mutant strains with higher aggregation in the late reproductive phases from the individual animals identified as positive for high levels of aggregation. We measured each aggregation mutant's lifetime and found mutants with noticeably shorter lifespans. New mutant strains that we have created have shorter lifespans and more PAB-1 protein aggregation. We also provide a novel strategy for forward genetic screening for ageing genes, which is applicable to additional ageing correlates or late onset symptoms and avoids suppression of reproduction.

DISCUSSION

In this work, we used image analysis and machine learning-based classification algorithms to precisely measure the tagRFP:PAB-1 reporter aggregation levels in several C. elegans populations. Determining if an aggregate exists is the biggest obstacle to correctly assessing aggregation. Protein aggregates are defined visually as sharp fluorescent puncta that are more intense than the overall level of protein expression in the pharynx. We were able to develop picture filtering processes based on the size, intensity, and pharyngeal background intensity of the item, as well as these visual identification criteria, which might detect the existence of protein aggregates. Although subjective categorization bias can affect hand annotation, the algorithms created here can reliably find aggregates in a variety of picture formats. These technologies allowed for quick, automated, on-chip measurement and sorting based on aggregation levels in populations of mutagenized worms, as well as the discovery of general increases in aggregation. Combining the outcomes of two machine learning methods enhanced the measurement of protein aggregation levels in the pharynx's darker areas, where aggregates accumulate. Identification is more difficult, but it has made it possible to recognise aggregates of various intensities in pictures with a variety of diffuse protein intensities and object sizes. Our aim was to utilise these metrics to distinguish between strains of baseline (wildtype) and greater aggregation, hence we opted to compute the areas and intensities determined by the classification algorithm (predicted) vs. the visually generated ground truth (actual) for our validation data. Despite the fact that we specifically trained our algorithm for the PAB-1 protein, this approach may be used to quantify other aggregating proteins.

In this work, we have devised a strategy for screening for

mutants that experience an increase in protein aggregation during the late reproductive stage, which results in mutants with a reduced lifetime. To keep age-synchronized parental worm populations that go through automated aggregation measurement and on-chip sorting, we created a semi-automated workflow using microfluidic lab-on-a-chip technology. This method makes it possible to perform a search that would otherwise be impossible since it would require manually classifying animals based on their visual phenotypes. These tests are quite labor-intensive and need several hours of physical effort. Phenotypic quantification can take considerably longer for high-resolution traits like PAB-1 protein aggregation. We intend to find the genes and pathways that control the aggregation and ageing networks in the C. elegans proteostasis network by additional analysis of identified aggregation and ageing mutants. The genetics of ageing can be better understood via screening, which might lead to a greater knowledge of how ageing impacts human health and how science and medicine can help people live longer, higher-quality lives.

REFERENCES

1. Zhou W, Sollie T, Tot T (2014). Breast cancer with neoductgenesis: histopathological criteria and its correlation with mammographic and tumour features. Int Journal of Breast Cancer. 101: 10-26.
2. Hermann G, Keller RJ, Drossman S (1999). Mammographic pattern of microcalcifications in the preoperative diagnosis of comedo ductal carcinoma in situ: histopathologic correlation. Canadian Asso of Radiologists Journal. 50: 235-240.
3. Tot T, Tabár L (2006). Mammographic–pathologic correlation of ductal carcinoma in situ of the breast using two- and three-dimensional large histologic sections. Seminars in Breast Disease. 8: 144-151.
4. Borgquist S, Zhou W, Jirström K (2015). The prognostic role of HER2 expression in ductal breast carcinoma in situ (DCIS); A Population-Based Cohort Study. BMC Cancer. 15: 210-225.
5. Shrout PE (1998). Measurement reliability and agreement in psychiatry. Statistical Methods in Med Research. 7: 301-317.
6. Barbarich Marsteller NC, Fornal CA, Takase LF (2013). Activity-based anorexia is associated with reduced hippocampal cell proliferation in adolescent female rats. Behav Brain Res. 236: 251-257.
7. Aoki, Chiye (2012). Adolescent female rats exhibiting activity-based anorexia express elevated levels of GABAA receptor α4 and δ subunits at the plasma membrane of hippocampal CA1 spines. Synapse. 66: 391-407.
8. Giordano GD, Renzetti P, Parodi RC (2001). Volume measurement with magnetic resonance imaging of hippocampus-amygdala formation in patients with anorexia nervosa. Journal of Endocrinological Investigation. J Endocrinol Invest. 24: 510-514.
9. Connan F, Murphy F, Connor SEJ (2006). Hippocampal volume and cognitive function in anorexia nervosa. Psychiatry Res. 146: 117-125.
10. Beadle JN, Paradiso S, Brumm M, Voss M, Halmi K et al (2015). Larger hippocampus size in women with anorexia nervosa who exercise excessively than healthy women. Psychiatry Res. 232: 193-199.